UNPAD

Indonesian Journal of Pharmaceutical Science and Technology
Journal Homepage : http://jurnal.unpad.ac.id/ijpst/

Simultaneous Estimation of Ketorolac Tromethamine and Tiemonium Methylsulphate in Biofluids by a Validated UPLC Method

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Submitted 26 October 2017; Revised 10 November 2017; Accepted 19 November 2017; Published 05 February 2018
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Abstract

An Ultra Performance Liquid Chromatography (UPLC) method has been developed for the simultaneous determination of ketorolac tromethamine (KTR) and tiemonium methylsulphate (TMS) in pharmaceutical and bio-samples. Calibration curves were linear with correlation coefficient 0.9997 at a studied concentration range of (0.1-10 µg mL^{-1}) for both drugs. The method was reproducible with relative standard deviation (RSD) for intraday precision was 0.32-0.99% for KTR and 0.40-1.01% for TMS and inter day precision was 0.51-2.77% for KTR and 0.41-2.26% for TMS. The mean recovery of the intraday assay was 99.18-103.34% for KTR and 99.14-101.76% for TMS and inter-day assay at six different days was 99.39-102.04% for KTR and 98.08-103.78% for TMS. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.125 and 0.41 µg mL^{-1}, respectively for KTR and 0.150 and 0.50 µg mL^{-1}, respectively for TMS. The method was successfully validated as per the ICH guidelines can be conveniently employed for routine quality control analysis of KTR and TMS in pharmaceutical formulations and biological samples without any interference.

Keywords: Ketorolac tromethamine, pharmaceutical and biosamples, tiemonium methylsulphate, UPLC

Estimasi Simultan Ketorolak Trometamin dan Tiemonium Metil sulfat dalam Cairan Bio dengan Metode UPLC Tervalidasi

Abstrak

Metode Kromatografi Cair Kinerja Ultra (UPLC) telah dikembangkan untuk penentuan simultan ketorolak trometamin (KTR) dan tiemonium metil sulfat (TMS) dalam farmasi dan bio-sampel. Kurva kalibrasi linear dengan koefisien korelasi 0,9997 pada rentang konsentrasi yang dipelajari (0,1-10 µg mL-1) untuk kedua obat tersebut. Metode itu direproduksi dengan standar deviasi relatif (RSD) untuk ketepatan pada hari yang sama adalah 0,32-0,99% untuk KTR dan 0,40-1,01% untuk TMS dan presisi antar hari adalah 0,51-2,77% untuk KTR dan 0,41-2,26% untuk TMS. Perolehan rata-rata dari pengujian hari yang sama adalah 99,18-103,34% untuk KTR dan 99,14-101,76% untuk TMS dan uji antar-hari pada enam hari yang berbeda adalah 99,39-102,04% untuk KTR dan 98,08-103,78% untuk TMS. Batas deteksi (LOD) dan batas kuantitasi (LOQ) masing-masing adalah 0,125 dan 0,41 µg mL-1 untuk KTR dan 0,150 dan 0,50 µg mL-1 untuk TMS. Metode ini berhasil divalidasi sesuai dengan pedoman ICH dapat dengan mudah digunakan untuk analisis pengendalian mutu rutin KTR dan TMS di formulasi farmasi dan sampel biologis tanpa gangguan apa pun.

Kata kunci: Farmasi dan biosamples, ketorolak trometamin, tiemonium metilsulfat, UPLC,

1. Introduction

Ketorolac tromethamine (KTR), 2-Amino-2-(hydroxyl methyl) 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 white crystalline powder and freely soluble in water, methanol and ethanol. 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 cylcooxygenase (COX-1) enzyme[2].

KTR is metabolized through hydroxylation in the liver to form p-hydroxy ketorolac and its metabolites are primarily excreted in the urine (91%), and the rest is eliminated in the feces. 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 events ranging from interstitial nephritis to complete kidney failure; hemorrhage, and hypersensitivity. Ketorolac also causes rise in serum transaminase levels. Allergic reactions (anaphylactoid reactions, asthma, bronchospasm, Stevens–Johnson syndrome, and toxic epidermal necrolysis) have been reported. Fluid retention and edema have been reported with the use of ketorolac and it should therefore be used with caution in patients with cardiac decompensation, hypertension or similar conditions[3,4].

Tiemonium methylsulphate (TMS) (Figure 2) 4-[3-Hydroxy-3-phenyl-3-(2-thienyl) propyl]-4-methyl morpholinium methylsulphate is quaternary ammonium antimuscarinics with peripheral effects similar to hose of atropine and are used in the relief of visceral spasms. It reduces muscle spasms of the intestine, biliary system, uterus & urinary bladder. It is indicated for the pain in gastrointestinal & biliary disease in the urology and gynecology such as gastroenteritis, diarrhea, dysentery, biliary colic, enterocolitis, cholecystytis, colonopathyes, mild cystitis, & spasmodic dysmenorrhea[5].

Adverse drug interactions in the concomitant use of ketorolac tromethamine and tiemonium methylsulphate are not well documented. This adverse interaction may effect on their plasma level. Their simultaneous determination in pharmaceutical formulation and biosamples is immense important. It was found that though individually these drugs have been analyzed by many methods[6,7,8,9,10] no one is available for their simultaneous estimation in a single run. Attempts have been made to develop new methods the estimation of KTR and TMS in pharmaceutical and biosamples. Chromatographic techniqus such as high-performance liquid chromatography (HPLC) with with diode array detection[11,12] and gas chromatography (GC) with electron capture detection have been used[13]. GC-MS methods using either electron impact (EI) or chemical ionization (CI) have been reported, but these procedures still require a tedious derivatization step prior to final analysis. However, liquid chromatography methods do not require a derivatization step.

In this paper UPLC method for simultaneous determination of ketorolac tromithamine and tiemoniummethylsulphate has been reported. The proposed method is optimized and validated according to ICH guidelines[14]. This simple, accurate, precise and sensitive method can also be used for the routine analysis of both drugs in mixture without time consuming.

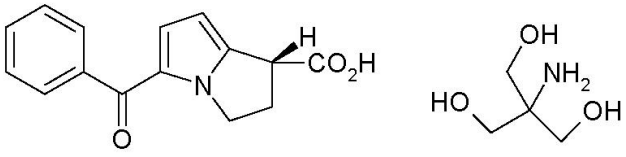

Figure 1. Structure of ketorolac tromethamine (MW=376.4)

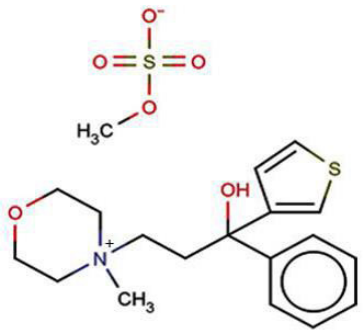

Figure 2. Structure of tiemoniummethylsulphate

2. Materials and Methods

2.1. Materials

HPLC-grade methanol was supplied by Sigma-Aldrich (Germany), Acetonitrile 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%.

Pharmaceutical formulations commercially available in Bangladesh were analysed to check the applicability of the method: Torax (10 mg) tablet by Square, Rolac (10 mg) tablet by Renata, Etorac (10 mg) tablet by Incepta, Zidolac (10 mg) tablet by Beximco, ketonic (10 mg) tablet by SK+F, Torax (30 mg) injection by Square, Rolac (30 mg) injection by Renata, Norvis (50 mg) tablet by Square, Visceralgin (50 mg) tablet by Nuvista, Timozin (50 mg) tablet by Incepta, Visrul (50 mg) tablet by Opsonin, Algin (50 mg) tablet by Renata, Visceralgin (5 mg) Injection by Nuvista, align (5 mg) Injection by Renata, align (10 mg) syrup by renata, visrul (10mg) syrup by opsonin. Biological samples, blood or urine (4 mL) were collected in bottles from the male patient under treatment with Algin after 1 hour of injection administration in Chittagong medical college and hospital, Bangladesh.

2.2. Preparation of Standards

Stock solutions of tiemonium methylsulphate were prepared at concentration level 100 µg mL^{-1} by dissolving an appropriate amount of each compound in ethanol and were stored at 4^{O}C, protected from light and used within 3 months. The stock solutions of drugs were further serially diluted daily before analysis with ethanol to make interim mixture solutions (controlled solution) at concentrations of 1, 3, 5, 7, 10 µg mL^{-1} for the compound. Buffer: 5 mM aqueous solution of dihydrogen sodium phosphate buffer was prepared by mixing appropriate weight in Milli Q water and filtered before use.

2.3. Sample Preparation

2.3.1. Pharmaceutical Samples

Twenty tablets were finely ground and powdered. A portion equivalent to 100 µg mL^{-1} solution was accurately weighed and transferred to volumetric flask and dissolved. Total volume made up to the mark diluting with ethanol. The solution was sonicated for 15 min and centrifuged at 3000 rpm for 10 min, and filtered through a 0.22 µm PTFE syringe filter with Whatman filter paper. An aliquot portion was transferred to volumetric flask, diluted with ethanol as to provide a stock solution of 100 µg mL^{-1}. All stock solutions were stored at 4^{O}C in refrigerator. Dilution has been made to accurately measured aliquots of the stock solution with ethanol to give working concentrations of the analyte.

2.3.2. Blood samples

0.5 mL upper layer of the whole blood after centrifugation were taken in each of three vials. 0.5 mL acetonitrile were added into each vial. For blank solution 1 mL ethanol were added into a vial and remaining two vials were spiked with the addition of 1 mL of 1 and 3 µg mL^{-1} standard solution. The solution

was sonicated for 15 min and centrifuged at 3000 rpm for 10 min, and filtered through a 0.22 µm PTFE syringe filter with Whatman filter paper. All solutions were stored at 4^{O}C in refrigerator before analysis.

2.3.3. Urine Samples

1 mL of urine were taken in each of three vials. For blank solution 1 mL ethanol were added into a vial and remaining two vials were spiked with the addition of 1 mL of 1 and 3 µg mL^{-1} standard solution. The solution was sonicated for 15 min and centrifuged at 3000 rpm for 10 min, and filtered through a 0.22 µm PTFE syringe filter with Whatman filter paper. All solutions were stored at 4^{O}C in refrigerator before analysis.

2.4. Preparation of Calibration Curve

Calibration curves were construced for five concentration levels of the analyte ranging from 1, 3, 5, 7 and 10 µg µL^{-1}. Peak area of each chromatogram at different levels was plotted against theoretical concentrations. Calibration curve constructed was fitted by a least squares linear regression to the equation, y = mx + c. where, y = response ratio, m = slope, x = concentration, c = intercept. With reference to this calibration equation unknown concentration of the analyte was determined.

2.5. Chromatographic Conditions

A standard solution of 5 µg mL^{-1} drug was used for the optimization of the chromatographic conditions. All through the experiment a reversed-phase Gemini 3U, C18, 110R (150 × 4.6 mm, 3 µm) column and NaH_2PO_4 (dihydrogen sodium phosphate, 5 mM) were used as buffer solution. Special attention have been paid on optimization of the mobile phase composition to gain good resolution avoding tailing of the peak. To detect the absorption maxima a UV scan of standard solution prepared by mobile phase was done in the range of 200 to 400 nm for the spectra of studied drugs (Figure 3). An efficient UPLC method was evaluated by the satisfactory results with good resolution at reduced elution time and tailing problems under this optimized composition. With respect to sharpness and symmetry of the peaks best flow rate was investigated. 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 1 mL/min at ambient temperature. The injection volume was 10 µL. Prior to the analysis buffer solution was filtered in vacuum using 0.2 µm membrane, mobile phase was degassed by a stream of helium and column was equilibrated with the mobile phase.

2.6. Validation Parameters

ICH guidelines were followed for the validation of the method[14]. In this regard analytical performance parameters precision, accuracy, specificity, limit of detection, limit of quantitation, linearity and range, suitability and robustness were studied.

3. Result and Discussion

3.1. Chromatography

The chromatographic conditions optimized were composition of the solvents, and mobile phase flow rate. Mobile phase it must elute all the different substances with satisfactory peak shape and in a short time. 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 with 5 mM dihydrogen sodium phosphate served our intentions best. Reversed-phase Gemini 3U, C18, 110R (150 × 4.6 mm, 3 µm) column and 5 mM NaH_2PO_4 as buffer solution all through the experimentwere used to study the simultaneous separation of both drugs. In particular, peak tailing observed was considerable. In order to determine the detection wavelength, the absorption spectra of all compounds were obtained. The absorption spectra of all compound showed absorption bands in the UV region with maximum absorption wave lengths between 235 to 245 nm as shown in. Therefore, 240 nm selected for monitoring as compromised to the both drug.

To determine the optimum mobile

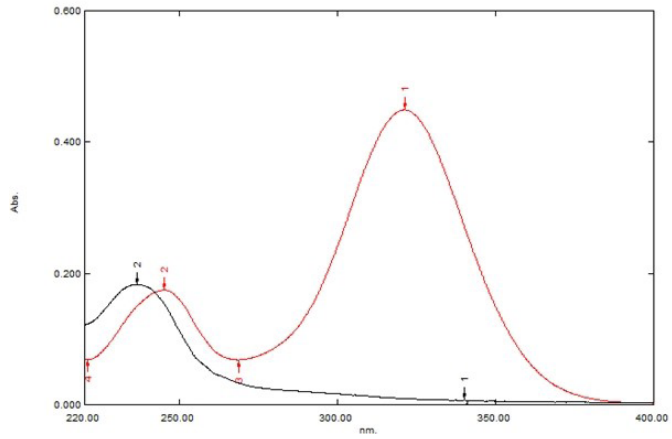

Figure 3. Electronic spectra of ketorolac tromethamine (red) and tiemoniummethylsulphate (black) standard solution in ethanol

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 1 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 5 mM NaH_2PO_4 by the composition of 90:05:05 (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 less than 2.2 min (2.191±0.005 minutes) (Figure 4).

The method developed herein was applied to various concentrations taken from the pharmaceutical products and plasma and urine samples for determining the content of investigated drugs.

Table 2 summarizes intraday and inter-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 and robustness are placed in Table 4. Table 5 also shows the column efficiency data as the validation evidences.

3.2. Validation parameters

3.2.1. Linearity

The calibration curves constructed for standard using working concentration at levels 1, 3, 5, 7, 10 µg mL^{-1} of each drug. Calibration curves were constructed using peak area of drug versus nominal concentrations of the analytes.

Calibration equations were y=23172x+2941.4 for KTR and y=14493x+3092.8 for TMS. The calibration curves were linear in the range of 1-10 µg mL^{-1} for both KTR and TMS. The coefficients of corelation (r^2) were 0.9997 for both drugs. Figure 5 shows the calibration curves for the determination of KTR and TMS, respectively.

3.2.2. Sensitivity

The limit of detection were calculated from calibration graph by the formula; LOD=3•Sxy/a, and the limit of quantification; LOQ=10•Sxy/a. The LOD and LOQ were found to be 0.125 and 0.41 µg mL^{-1} for KTR and 0.150 and 0.50 µg mL^{-1} for TMS respectively. These results indicate that

Table 1. Optimum gradient program for the proposed method

Time (min)	Solvent Composition			Flow rate mL/min	R time (min)
	CH_3CN	CH_3OH	5mM NaH_2PO_4		
0.01-3.0	5	90	5	1	1.15 for TMS 2.15 for KTR

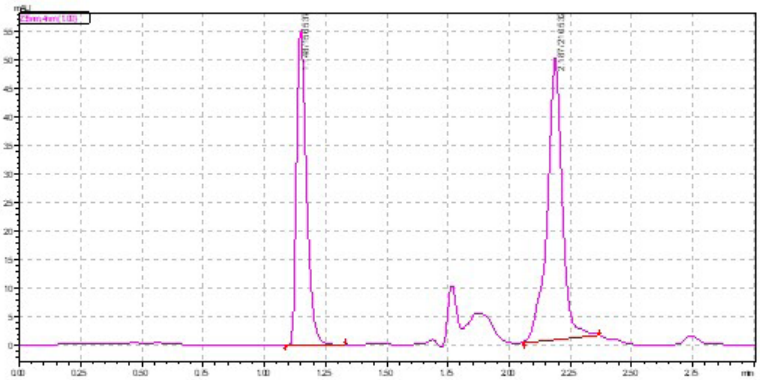

Figure 4. Typical UPLC chromatogram of the examined drug (10 µg mL^{-1}) in Chromatographic conditions described in text. Peaks: 1.148 min (TMS), 2.187 (KTR)

method was sensitive enough for therapeutic assay.

3.2.3. Recovery/Accuracy

The results of recovery obtained from the within-day assay at five concentrations (n=5) by the proposed method was 99.18-103.34% for KTR and 99.14-101.76% for TMS while between-day assay at six different days was 99.39-102.04% for KTR and 98.08-103.78% for TMS. The recovery showed that high accuracy of the drug determination. Intraday and inter-day recovery data of proposed method are presented in Table 2.

3.2.4. Precision

The relative standard deviations (RSD) obtained for the within-day assay at five concentrations (n=5) was 0.32-0.99% for KTR and 0.40-1.01% for TMS while between-day assay was in the range 0.51-2.77% for KTR and 0.41-2.26% for TMS. The precision results showed that the high precision of the method. Intraday and inter-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 showed that drugs were free of interference from potential impurities and degradation products by the absence of any peak in the same retention times. Peak purity of KTR and TMS was passed in standard. From the chromatogram shown in Figure 4, it is evident that under the chosen chromatographic conditions KTR (Tr=2.187 min), TMS (Tr=1.148 min). Results indicate the high specificity of the method and can be

Table 2. Summarizes intraday and inter-day precision and accuracy data

Analysis	Conc. Added µg mL^{-1}	KTR			TMS		
		Conc. Found µg mL^{-1}	RSD (%)	Recovery (%)	Conc. Found µg mL^{-1}	RSD (%)	Recovery (%)
Intra day	1	1.0±0.01	0.99	100.26	0.99±0.01	1.01	99.17
	3	3.10±0.02	0.65	103.34	2.97±0.02	0.67	99.14
	5	4.96±0.023	0.46	99.18	5.09±0.03	0.59	101.76
	7	7.09±0.03	0.42	101.3	7.05±0.04	0.57	100.72
	10	10.08±0.03	0.32	100.78	9.94±0.04	0.40	99.43
Inter day	1	0.98±0.019	1.98	101.09	1.04±0.023	2.26	98.08
	3	3.110.022	0.71	96.39	2.93±0.021	0.73	103.78
	5	4.96±0.025	0.51	102.04	5.09±0.029	0.58	99.18
	7	6.87±0.088	1.29	100.87	7.25±0.034	0.47	98.19
	10	9.93±0.27	2.77	99.45	9.94±0.04	0.41	99.32

Table 3. Validation performance of the proposed UPLC method

Validation Parameters	KTR	TMS
Measurement wavelength (nm)	240	240
Linear range (µg mL^{-1})	0.2-10	0.5-10
Linearity equation	y=23172x+2941.4	y=14493x+3092.8
Standard deviation of the slope	607.5	403.2
Correlation coefficient (r)	0.9997	0.9997
Relative standard deviation (% RSD) Intraday Inter day	0.32-0.99 0.51-2.77	0.40-1.01 0.41-2.26
Relative standard deviation (% R) Intraday Inter day	99.18-103.34 99.39-102.04	99.14-101.76 98.08-103.78
Limit of detection, LOD (µg mL-1)	0.125	0.150
Limit of quantification, LOQ (µg mL^{-1})	0.41	0.50

used in a stability assay and routine analysis of the investigated drugs.

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. Variation in the experimental parameters (flow rate, isocratic program) provided an indication of its reliability during normal use and concluded that the method was robustas 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 and TMS to be performed. The system suitability was assessed by replicate injections (n=5) of the sample at 5 µg mL^{-1} 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 0.49% for KTR, 0.56% for TMS and that of retention time 0.17 min for KTR and 0.37 min for

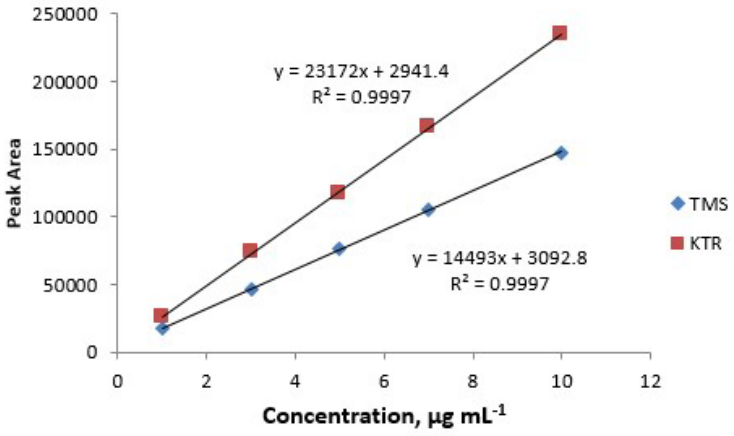

Figure 5. Calibration curve for the determination of KTR and TMS

Table 4. Validation parameters in terms of suitability and robustness

Conc. (5 µg mL^{-1})		Suitability	
		R.T(n=5)	Area(n=5)
KTR	Average	2.191±0.0037	116828±578
	RSD (%)	0.17	0.49
TMS	Average	1.149±0.004	76694.33± 430
	RSD (%)	0.37	0.56

TMS 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 of theoretical plate number, tailing factor, separation factor, resolution factor and capacity factor as shown in Table 5 revealed the excellent performance of analytical column.

3.3. Applications

3.3.1. Pharmaceutical formulations

The method developed here was applied to various concentrations (3.0, 5.0 µg mL^{-1}) of solutions prepared from pharmaceutical products for determining the content of KTR and TMS. The values of the overall drug percentage recoveries and the RSD values of measurements are as presented in Table 6 and 7.

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. 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.

3.3.2. Biosamples

The method developed here was applied to various spiked concentration of solutions prepared from biological samples, plasma and urine, taken from one volunteer under regular treatment for determining the content of KTR and TMS. Standard addition method

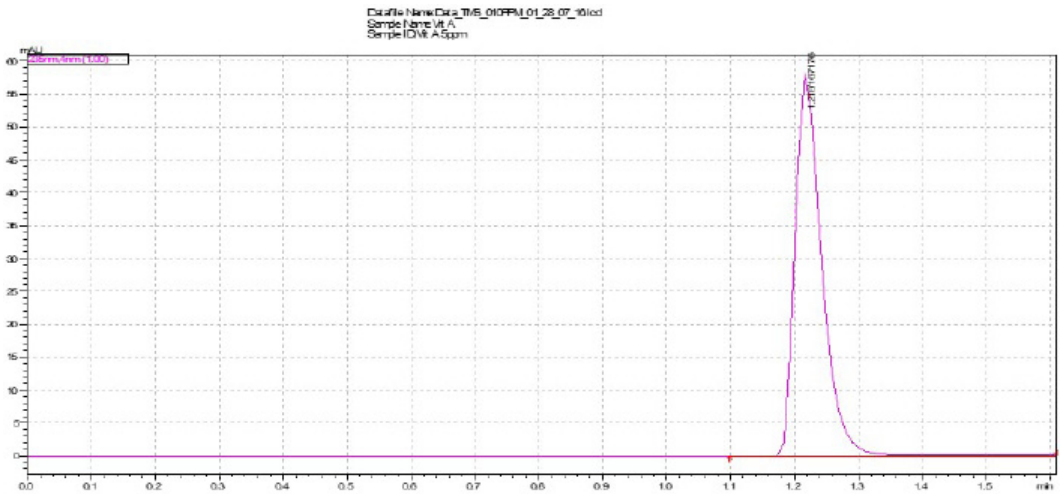

Figure 6. Typical UPLC chromatogram of the TMS (10 µg mL^{-1}) at retention time of 1.2 min in standard

Table 5. Validation parameters in term of column efficiency

Validation parameters	For KTR		For TMS	
	Average	RSD	Average	RSD
NTP	4928.67±34.5	0.7	3355.33±60.5	1.8
HETP	30.59±0.47	1.56	1.45±0.009	10.30
T.F.	0.87±0.01	1.53	46.34±4.7	0.63
C.F, k′	0.89±0.003	0.36	0.45±0.001	0.225
Resolution factor		9.94		
Separation factor		1.40		

was applied to the analysis of biological samples. Regression equation after standard addition yielded both drugs as shown in Table 8 and Table 9. The values of the overall drug percentage recoveries and the RSD values of measurements are presented. The absence of any endogenous interfering peak observed in the extracts of bio-fluids overlapping with any analyte indicates the high specificity of the method which can be used in therapeutic and routine analyses.

3.3.3. Drug Intraction Study

The drug interaction for the concomitant use of KTR and TMS was studied few. No evidences of degradation or interferences was detected at present conditions with in study range or retention time as shown in Figure 6-8. From the chromatogram it is evident that no drug interactions or online derivatization has been occurred in their combined determination, since there was no peaks on their retention times. Peak purity was passed in standard as the excellent RSD precision of area to retention time and area of 10 measurements of 5 μg mL^{-1} KTR and TMS. The average retention time for TMS in single measurement of which was 1.14 ± 0.04 min and RSD was 0.34%. The average retention time for KTR in single measurement of which was 2.191 ± 0.0037 min and RSD was 0.17%. RSD of area of ten measurements

Table 6. Determination of Ketorolac in pharmaceutical formulation by the proposed method

Brand Name	Conc.±SD (μg mL^{-1})		RSD (%)	Recovery (%)	Average Recovery (%)
	Added	Found			
Tablet					
Etorac	3	2.28±0.007	0.008	76.19	75.33
	5	3.78±.05	0.35	74.47	
Ketonic	3	1.86±0.1	2.43	62.21	61.96
	5	3.08±0.08	0.67	61.72	
Rolac	3	1.98±0.02	0.31	66.17	66.16
	5	3.30±0.04	0.32	66.14	
Xidolac	3	2.50±0.008	0.09	83.43	85.13
	5	4.34±0.01	0.07	86.83	
Torax	3	2.05±0.1	1.43	68.37	67.41
	5	3.32±0.03	0.22	66.44	
Injection					
Torax	3	2.33±0.1	1.25	77.82	76.95
	5	3.80±0.08	0.59	76.07	
Rolac	3	1.63±0.06	0.95	54.47	54.34
	5	2.71±0.007	0.07	54.21	

Table 7. Determination of Ketorolac in pharmaceutical formulation by the proposed method

Brand Name	Conc.±SD (µg mL^{-1})		RSD (%)	Recovery (%)	Average Recovery (%)
	Added	Found			
Tablet					
Algin	3	2.18±0.08	2.17	72.71	72.56
	5	3.62±0.19	3.21	72.41	
Visceralgin	3	2.34±0.03	0.73	77.99	76.06
	5	3.71±0.01	0.23	74.14	
Visrul	3	2.28±0.005	0.12	75.96	76.06
	5	3.81±0.04	0.65	76.16	
Timozin	3	2.29±0.005	0.14	76.37	73.88
	5	3.56±0.001	0.02	71.39	
Norvis	3	2.62±0.03	0.85	87.52	86.41
	5	4.26±0.02	0.31	85.31	
Injection					
Algin	3	2.14±0.03	0.29	71.41	71.13
	5	3.54±0.06	0.07	70.85	
Visceralgin	3	2.39±0.04	0.12	67.78	69.63
	5	3.57±0,06	0.43	71.49	
Syrup					
Algin	3	2.29±0.03	0.39	76.37	75.44
	5	3.72±0.06	0.09	74.51	
Visrul	3	2.17±0.03	0.98	72.38	69.25
	5	3.30±0.05	0.17	66.13	

was 0.49% and 0.56% for KTR and TMS, respectively. In mixture the RSD of average retention time and peak area were almost same as that of individual values. Our present study shows that both drugs have been determined simultaneously in their mixture free of interference from potential impurities and degradation products by the absence of any peak in the same retention times.

4. Conclusion

The proposed work provides a fast, precise, sensitive, accurate, linear, robust, simple and rugged UPLC assay method. For the proposed method both the drugs gave well define peaks. They were well separated. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. The reproducibility,

Table 8. Determination of TMS in pharmaceutical formulation by the proposed method

Bio-samples	Drug (Injection)	Conc. (µg mL^{-1})			RSD (%)	Recovery (%)
		Spiked	Found	Samples		
Plasma	Algin	0.0	0.94±0.03	0.94	3.01	--
		1.0	1.95±0.006	0.95	1.71	108.43
		3.0	3.94±0.02	0.94	4.99	99.97
Urine	Algin	0.0	0.54±0.02	0.54	1.95	--
		1.0	1.51±0.03	0.51	1.00	93.10
		3.0	3.52±0.02	0.52	1.64	95.28

Table 9. Determination of Tiemoniummethylsulphate in biological sample by the proposed method.

Bio-samples	Drug (Injection)	Conc. (µg mL^{-1})			RSD (%)	Recovery (%)
		Spiked	Found	Samples		
Plasma	Algin	0.0	0.81±0.02	0.81	1.68	--
		1.0	1.83±0.05	0.83	1.74	102.2
		3.0	3.82±0.04	0.82	0.66	101.9
Urine	Algin	0.0	0.06±0.003	0.061	1.81	--
		1.0	1.06±0.008	0.061	0.45	99.8
		3.0	3.06±0.006	0.060	0.11	98.4

repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation in comparison to previous methods. No evidences of drug interaction has been detected. Method was successfully validated as per ICH guidelines can be conveniently employed for routine quality control analysis of ketorolac tromithamine and tiemonium methylsulphate in pharmaceutical formulation and biological samples without any interference.

5. Conflicts of interest

Authors declared no conflicts of interest.

References

1. Rang H, Dale M, Ritter J, Flower R. Pharmacology, 6th Ed., Churchill Livingstone, Elsevier. 2007;227-30,
2. Wang Z, Dsida R, Avram M. Determination of ketorolac in human plasma by reversed – phase high-performance liquid chromatography using solid-phase extraction and ultraviolet detection, J. Chromatography B. 2001;755(1-2):383-6.
3. Shankar C, Mishra M. Development and in-vitro evaluation of gelatin A microspheres of Ketorolac tromethamine for intranasal administration. Acta Pharm. 2003;53;101–10.
4. Shyamala B. and Sanmathi B.S., Poly(lactic acid) microspheres of Ketorolac tromethamine for parenteral controlled drug delivery system. Indian J Pharm Sci. 2001;63; 538–40.
5. Bejjani A, Nsouli B, Zahraman K, Assi re-

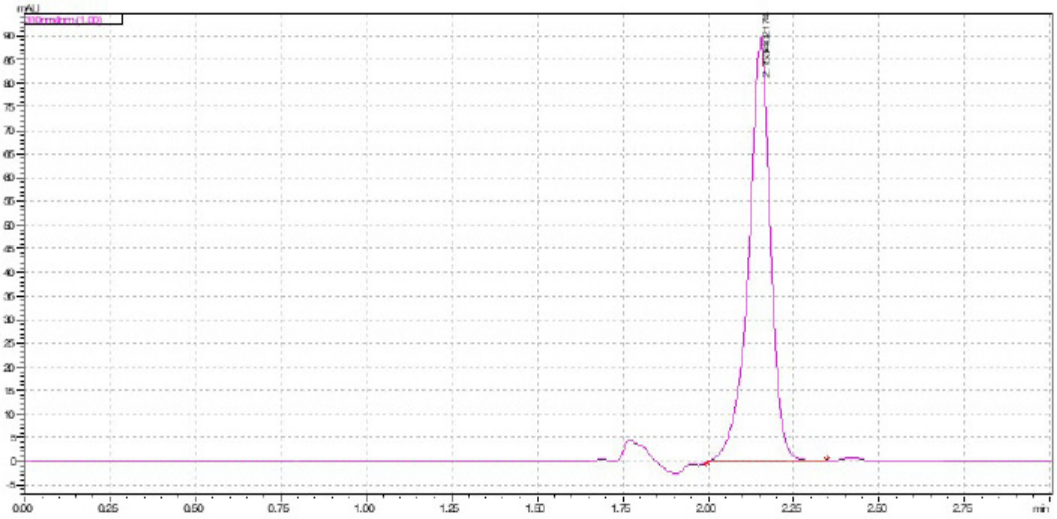

Figure 7. Typical UPLC chromatogram of the KTR (10 µg mL^{-1}) at retention time of 2.15 min in standard

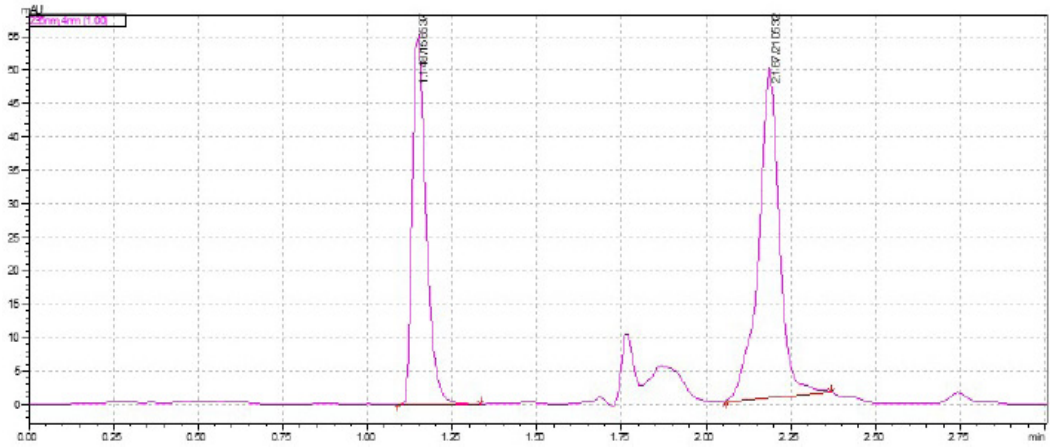

Figure 8. Typical UPLC chromatogram of the KTR and TMS (10 µg mL^{-1}) at retention time of 1.14 min for TMS and 2.18 for KTR in standard

peatability and accuracy of the proposed method were found to be satisfactory which S, Younes G., Yazbi F. Swift Quantification of Fenofibrate and Tiemoniummethylsulfate Active Ingredients in Solid Drugs Using Particle Induced X-Ray Emission. Advanced Materials Research, 2011;324;318-23.

6. Islam MS, Wahiduzzaman, Islam MS, Rafiquzzaman M, Kundu SK. UV-Spectroscopic method for estimation of Tiemoniummethylsulfate 50 mg tablet in bulk and pharmaceutical preparations. Int J Pharm Sci Res. 2014;5(2):548-55.
7. Ayad M, El-Balkiny M, Hosny, M Metias Y. Spectrophotometric Determination of Tiemonium Methyl Sulfate, Itopride Hydrochloride and Trimebutine Maleate via Ion Pair Complex Formation and Oxidation Reaction. Indian J. of Advances in Chem. Sci. 2016;4(1):85-97.
8. Zaaza HE, Abbas SS, EL-Sherif ZA, El-Zeany B, EL-Haddad DA. Stability indicating spectrophotometric methods for determination of tiemoniummethylsulphate in the presence of its degradation products. J. App. Pharm. Sci. 2014;4(1); 33-45.
9. Ramadan NK, Abd El Halim LM, El Sanabary HFA, Salem MY. Stability indicating spectrophotometric methods for the determination of TiemoniumMethylsulphate. Int. J. Drug Dev. & Res. 2014;6(1); 160-8.
10. Ramadan NK, Abd El Halim LM, El Sanabary HFA, Salem MY. Stability Indicating Chromatographic methods for the Determination of Tiemoniummethylsulphate, Int. J. of Advanced Res. 2014;2(1); 366-76.
11. Sunil G, Jambulingam M, Thangadurai SA, Kamalakannan D, Sundaraganapathy R, Jothimanivannan C. Development and validation of Ketorolac Tromethamine in eye drop formulation by RP-HPLC method. Arabian Journal of Chemistry. 2017;10;S928–S935.
12. Uddin MN, Das S, Khan SH, Shill SK, Bhuiyan HR, Karim R. A novel validated uplc method for the estimation of ketorolac tromethamine in pharmaceutical formulation. Research. 2014;1:1237.
13. Ayad M, El-Balkiny M, Hosny M, Metias Y. Green Validated method for determination of tiemonium methyl sulfate using reversed-phase high-performance liquid chromatography technique with stability-indicating studies Innovare J. of Medical Sci. 2016;4(3);1-9.
14. ICH Harmonized Tripartite (1996) Validation of Analytical Procedures: Text and Methodology Q2 (R1), Current Step 4 Version, Parent Guidelines on Methodology Dated November 6 1996, Incorporated In: International Conference on Harmonisation. Geneva, Switzerland; 2005.