European Journal of Chemistry

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A mathematical expression for tiemonium methylsulphate in its simultaneous spectrophotometric estimation with ketorolac tromethamine

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RESEARCH ARTICLE

10.5155/eurjchem.9.2.138-146.1715

Received: 11 May 2018
Received in revised form: 25 May 2018
Accepted: 25 May 2018
Published online: 30 June 2018
Printed: 30 June 2018

KEYWORDS

Bio-samples
Mathematical expression
Ketorolactromethamine
Tiemoniummethylsulphate
Pharmaceutical formulation
Spectrophotometric and UPLC method

ABSTRACT

Attempt has been made to develop a new, accurate, precise and economic spectrophotometric method for the simultaneous determination of ketorolactromethamine (KTR) and tiemoniummethylsulphate (TMS) in pharmaceutical formulation and bio-samples. It is noted that KTR shows two absorption peaks at 320 and 245 nm whereas TMS shows maximum absorption at 235 nm. In a mixture solution, peaks at 245 nm for KTR and at 235 nm for TMS are merged into a single peak at 240 nm. Hence KTR might be determined using its calibration equation constructed at 320 nm but the determination of TMS alone in their mixture measuring its absorption at 240 nm is difficult. Therefore, for the determination of TMS, a mathematical expression $x_T = k_1.y_{K+T} - k_2.y_K + k_3$ (y = Absorbance, x = Concentration) has been derived. This expression will give its concentration in mixture without having its absorption at 240 nm. Method has been applied to pharmaceutical and bio-samples successfully. Results have been compared to that estimated by new UPLC method developed as to validate this spectrophotometric method. The LOD and LOQ were found to be 0.25, 0.80 µg/mL for KTR and 0.31, 0.95 µg/mL for TMS, respectively.

Cite this: *Eur. J. Chem.* **2018**, *9(2)*, 138-146 **Journal website**: www.eurjchem.com

1. Introduction

Ketorolac tromethamine, (±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid with 2-amino-2-(hydroxyl methyl)-1,3-propanediol (1:1) (Figure 1), is a member of non-steroidal anti-inflammatory drug (NSAID) family. It shows potent prostaglandin cyclooxygenase inhibitory activity. Ketorolac, when administered intramuscularly or orally, is a safe and effective analgesic agent for the short-term management of acute postoperative pain and can be used as an alternative to opioid therapy [1]. It has been investigated extensively for use in post-operative analgesia both as a sole agent and supplement opioid analgesics and excellent applicability in the emergency treatment of postoperative cancer pain and in the treatment of migraine pain [2]. An ophthalmic solution of ketorolac is available and is used to treat eye pain and to relieve the itchiness and burning of seasonal allergies. Ketorolac should be avoided in patients with renal disfunction. The patients at highest risk, especially in the elderly, are those with fluid imbalances or with compromised renal function. Ketorolac is not recommended for long-term chronic pain or for pre-operative analgesia. Since this drug is widely seen in clinical cases, the measurement in samples continues to be of concern and investigation [3,4].

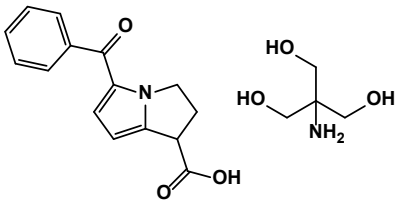

Figure 1. Structural formulae for ketorolac tromethamine.

Chemically tiemonium methylsulphate is known as 4-(3-hydroxy-3-phenyl-3-(2-thienyl)propyl)-4-methyl morpholinium methylsulphate (Figure 2). Tiemonium methylsulphate is an antispasmodic agent that stabilizes the cell membrane of the GI tract by strengthening calcium bonding with phosphorlipids and proteins. Tiemonium methylsulphate should not be used in glaucoma, disorders of prostate or bladder, tachycardia, myocardial infarction, paralytic ileus, pyloric stenosis and acute oedema of the lung. Due to the risk of agranulocytosis related to noramidopyrine, the use of this drug is not recommended in pregnant women. There is risk of anticho-

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.9.2.138-146.1715

linergic effects in infants at therapeutic doses because it decreases milk secretion and diffuse into milk [5]. In addition, both have their adverse effect due to their abuse or overdose. Therefore, their regular monitoring is immense important.

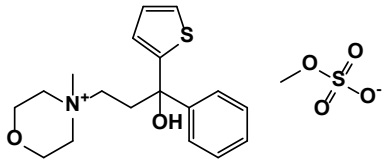

Figure 2. Structure of tiemonium methylsulphate.

In view of the above considerations, versatile analytical procedure is needed to assay the drugs in a setting where a patient used them. In recent years a large number of methods are reported for individual estimation of either KTR or TMS [6-10]. But no method is available for their simultaneous estimation in mixture in case of the concomitant administration of the both drugs. Therefore a single, rapid, reliable method with high precision is immense necessitated for simultaneous estimation of ketorolac tromethamine and tiemonium methylsulphate. Spectrophotometer is available in all laboratories and chromatographic methods (HPLC and GC) [11-17] analysis require more expenses. Hence spectrophotometric method will be universal one especially for the countries like us where there is scarcity of budgets. Attempts have been made to develop new spectrophotometric method for the estimation of KTR and TMS in pharmaceutical and bio-samples. An ultra-performance liquid chromatography method was also developed for simultaneous estimation of KTR and TMS in mixture and results obtained by the spectrophotometric method have been compared.

2. Experimental

2.1. Instrumentation

A Shimadzu UV Visible UV-1800 spectrophotometer model with suitable settings equipped with 1 cm quartz cells was used for absorbance. The spectral band length was 1 nm, the wavelength accuracy was 0.5 nm with automatic wavelength correction, and the recorder was a computer-controlled in the wavelength range 190-1100 nm.

2.2. Ultra-performance liquid chromatography (UPLC)

A Shimadzu (Tokyo, Japan) binary low-pressure gradient system was used for the chromatographic determination of the examined analyte where the solvent lines were mixed in an FCV-20AH2 mixer equipped with two Nexera LC-30AD pumps to deliver the mobile phase. SPD-M20A Photodiode Array Detector, complied with data acquisition software Lab Solutions-Nexera PDA by Shimadzu was used. The analytical column, Gemini 3U, C18, 110R (150 × 4.6 mm, 3 µm), was purchased from Phenomenex (Torrance, USA).

2.3. Materials

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%.

Pharmaceutical formulations commercially available in Bangladesh were analyzed 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 (10 mg) syrup by opsonin. Biological samples were collected from Chittagong Medical College and Hospital, Chittagong, Bangladesh

2.4. Preparation of standards

Stock solutions of ketorolac tromethamine and tiemonium methylsulphate were prepared at concentration level 100 µg/mL by dissolving an appropriate amount of each compound in ethanol and were stored at 4 °C, protected from light and used within three 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 and 10 µg/mL for the compound. Buffer: 5 mM aqueous solution of dihydrogen sodiumphosphate buffer was prepared by mixing appropriate weight in Milli Q water and filtered before use.

2.5. Sample preparation

2.5.1. Pharmaceutical samples

Twenty tablets or the content were finely ground and powdered. An accurately weighed portion equivalent to 100 µg/mL solution for each compound, was transferred to volumetric flask, dissolved and diluted up to the mark 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. All stock solutions were stored at 4 °C in refrigerator. Dilution has been made to accurately measured aliquots of the stock solution with ethanol to give working concentrations of the analyte.

2.5.2. Biological samples

2.5.2.1. Blood samples

Regarding human blood (4 mL) was collected in bottles from the affected persons. Upper layer of the blood (0.5 mL) were taken in three vials. Acetonitrile (0.5 mL) were added into each vial. For blank solution 1 mL ethanol were added into one vial. 1 mL of 1 µg/mL and 3 µg/mL standard solutions were added into remaining two vial. 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 °C in refrigerator.

2.5.2.2. Urine samples

Regarding human urine (20 mL) was collected in bottles from the affected persons. Urine (1 mL) was taken in three vials. For blank solution 1 mL ethanol were added into one vial. 1 mL of 1 µg/mL and 3 µg/mL standard solutions were added into remaining two vial. 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 °C in refrigerator.

2.6. Solvent selection

To optimize the solvent absorption spectra of the drugs were recorded in water, methanol and ethanol. Based on the peak shape best solvent was selected. Spectra of drugs were also recorded in solvent mixture at different ratio.

2.7. Preparation of calibration curve

Calibration curves were prepared for five concentration levels ranged from 1-10 µg/mL of each analyte for standard mixture. The calibration curves were constructed by plotting peak area or absorbance 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 concentrations of KTR were determined with reference to the calibration equation.

2.8. Derivation of an equation for the calculation TMS in mixture

From calibration equation, the absorbance of KTR in mixture at λ_{320} (nm) is written by

$$y_K = m_1 x_k + c_1 \quad (1)$$

where, y_K = Absorbance of KTR at 320 nm; x_k = Concentration of KTR; m_1 = Slope of the straight line; c_1 = Intercept of the straight line.

The absorbance of KTR at λ_{245} nm,

$$y_2 = m_2 x_k + c_2 \quad (2)$$

where, x_k = Concentration of KTR, y_2 = Absorbance of KTR at 245 nm.

The absorbance of TMS at λ_{235} nm,

$$y_T = m_3 x_T + c_3 \quad (3)$$

$$x_T = \frac{y_T - c_3}{m_3} \quad (4)$$

where, x_T = Concentration of TMS, y_T = Absorbance of TMS at 235 nm.

The total absorbance of KTR and TMS in mixture at λ_{240} nm

$$y_{K+T} = y_2 + y_T \quad (5)$$

From Equation (1), it is found,

$$X_k = \frac{y_K - c_1}{m_1} \quad (6)$$

Putting the value of x_k in Equation (2),

$$y_2 = \frac{m_2}{m_1}(y_K - c_1) + c_2 \quad (7)$$

From Equation (5), it is written,

$$y_T = y_{K+T} - y_2 \quad (8)$$

$$y_T = y_{K+T} - \left\{\frac{m_2}{m_1}(y_K - c_1) + c_2\right\} \quad (9)$$

$$y_T = y_{K+T} - \frac{m_2}{m_1}(y_K - c_1) - c_2 \quad (10)$$

From Equation (4), concentration of TMS in mixture is written by

$$x_T = \left\{y_{K+T} - \frac{m_2}{m_1}(y_K - c_1) - c_2 - c_3\right\} / m_3 \quad (11)$$

$$x_T = \frac{1}{m_3}\left\{y_{T+K} - \frac{m_2}{m_1}(y_K - c_1) - c_2 - c_3\right\} \quad (12)$$

$$x_T = \frac{1}{m_3 m_1}\{y_{T+K} m_1 - m_2(y_K - c_1) - m_1 c_2 - m_1 c_3\} \quad (13)$$

$$x_T = \frac{y_{T+K}}{m_3} - \frac{m_2(y_K - c_1)}{m_3 m_1} - \frac{c_2}{m_3} - \frac{c_3}{m_3} \quad (14)$$

$$x_T = \frac{1}{m_3} y_{T+K} - \frac{m_2}{m_3 m_1} y_K + \left(\frac{m_2 c_2}{m_3 m_1} - \frac{c_2}{m_3} - \frac{c_3}{m_3}\right) \quad (15)$$

$$x_T = k_1 y_{K+T} - k_2 y_K + k_3 \quad (16)$$

From Equation (16) the concentration of tiemonium methylsulphate in mixture can be calculated. Where, $k_1 = \frac{1}{m_3}$, $k_2 = \frac{m_2}{m_3 m_1}$ and $k_3 = \left(\frac{m_2 c_2}{m_3 m_1} - \frac{c_2}{m_3} - \frac{c_3}{m_3}\right)$.

2.9. Chromatographic conditions

An efficient HPLC method previously developed [16] was applied throughout the experiment to monitor both drugs. Chromatography was performed under isocratic condition at ambient temperature using the mobile phase composed of buffer (5): CH_3OH (90): ACN (5): 0.05 M NaH_2PO_4. The DAD detection at wavelength of 235 and 320 nm were found to be suitable to monitor the column effluent.

2.10. Validation parameters

Analytical performance parameters; precision, accuracy, specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity and range, suitability and robustness was studied for the validation of the method [18].

3. Results and discussion

3.1. Absorption spectra

The absorption spectra of the drugs are recorded in the wavelength 200-400 nm. The typical superimposed UV spectra of ketorolac tromithamine and tiemonium methylsulphate in ethanol are presented in Figures 3-5.

3.2. Effect of solvent

Ketorolac tromethamine and tiemonium methylsulphate were freely soluble in water, methanol and ethanol. The typical UV spectra of ketorolac tromithamine and tiemonium methylsulphate in ethanol are presented in Figures 6-8.

The absorption peak of KTR and TMS in water solution were not fair. In methanolic solution KTR gave a good absorption peak but TMS did not give a well absorption peak.

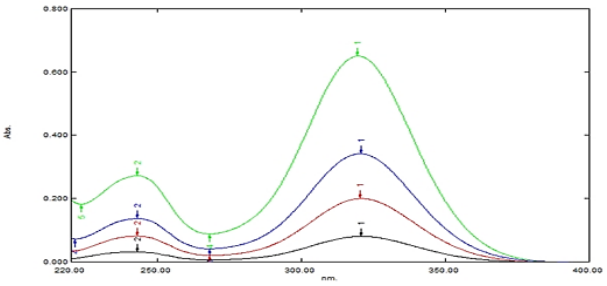

Figure 3. Representative UV spectrum of ketorolac tromithamine in ethanol at λ_{max} = 320 nm and λ_{max} = 245 nm.

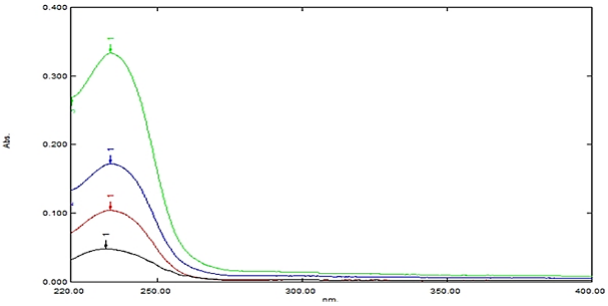

Figure 4. Representative UV-spectrum of tiemonium methylsulphate in ethanol at λ_{max} = 235 nm.

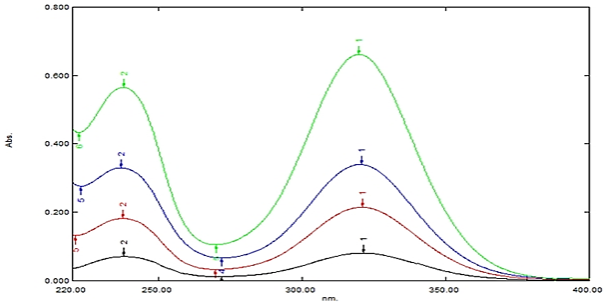

Figure 5. UV-spectra of ketorolac tromithamine and tiemonium methylsulphate in mixture at λ_{max} = 320 nm for KTR and λ_{max} = 240 nm for KTR+TMS.

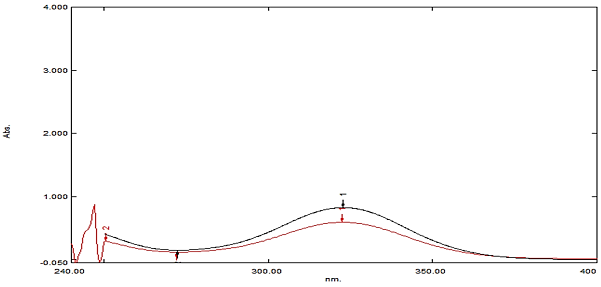

Figure 6. UV-spectrum of ketorolac tromithamine and tiemonium methylsulphate in water.

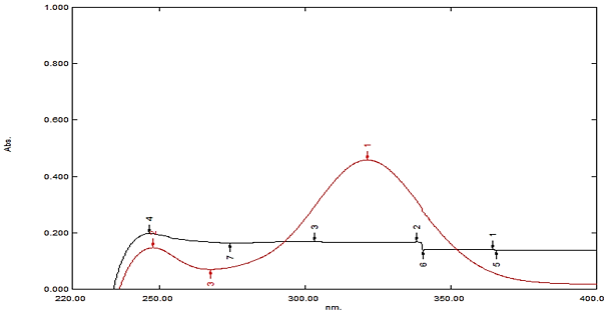

Figure 7. UV-spectrum of ketorolac tromithamine and tiemonium methylsulphate in methanol.

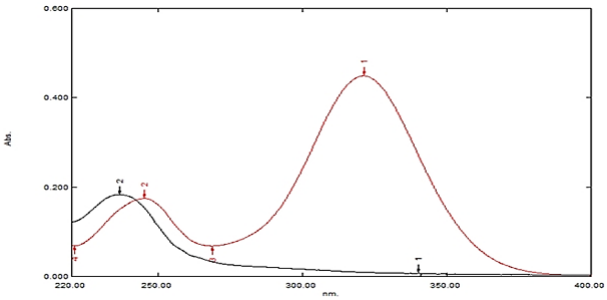

Figure 8. UV spectra of ketorolac tromithamine (red) and tiemonium methylsulphate (black) in ethanol.

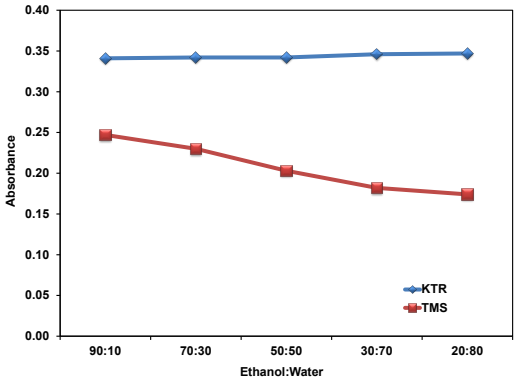

Figure 9. Effect of solvent ratio (ethanol:water) on absorbance of TMS and KTR.

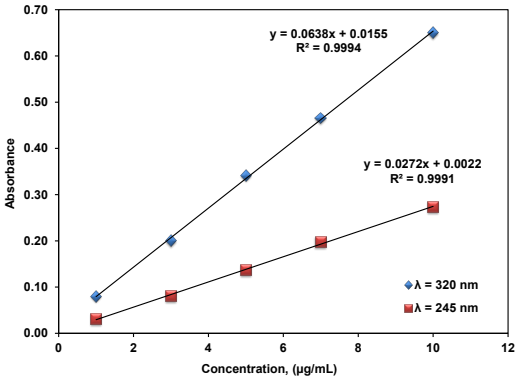

Figure 10. Calibration curves for ketorolac tromithamine (01-10 µg/mL) at λ = 320 nm and λ = 245 nm.

In ethanolic solution ketorolactromethamine and tiemonium methylsulphate gave good absorption peak. In view of the above consideration, ethanol was selected as a solvent. The absorbance of ketorolac tromithamine was almost same at different ethanol and water ratio but the absorbance of tiemonium methylsulphate was changed at different ethanol and water ratio. Absorbance of TMS was decreased with increased the percentages of water in ethanol as shown in Figure 9.

3.3. Method validation

Method was validated in terms of ICH [18] analytical performance parameters; precision, accuracy, specificity, limit of detection, limit of quantitation, linearity and range, suitability and robustness. Results obtained by UV Spectrophotometric method and Equation (16) were compared to that obtained by UPLC method as to validate the spectrometric method.

3.4. Calibration curve for ketorolac tromethamine at 320 and 245 nm

The calibration curves were made as described in the experimental procedure and correlation coefficients for maximum absorbance at 320 and 245 nm were found 0.9994 and 0.9991, respectively. It was constructed by plotting absorbance against corresponding concentrations for five standard solutions containing 1-10 µg/mL of KTR according to the general procedure. The calibration curves are shown in Figure 10 for KTR determination.

The linearity range, regression equation and correlation coefficient were obtained by the method of least squares, y = slope (m) × concentration (x) + intercept (c). Unknown concentration of the analyte was determined with reference to the calibration equation. The line plot between the absorbance and the amount KTR was drawn and the straight line obeyed the equation $y = 0.0638x + 0.0155$ of maximum absorbance at 320 nm and $y = 0.0272x + 0.0022$ of maximum absorbance at 245 nm for KTR, having regression coefficient of r^2 0.9994 and 0.9991, respectively.

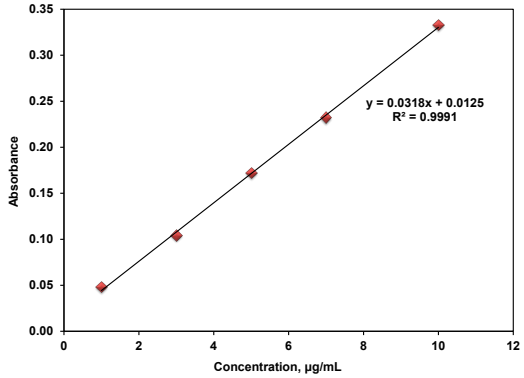

Figure 11. Calibration curve for tiemonium methylsulphate (01-10 µg/mL) at λ = 235 nm.

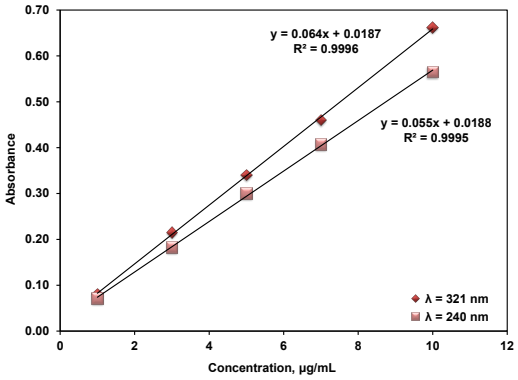

Figure 12. Calibration curve for different KTR and total of TMS in mixture at concentrations (01-10 µg/mL) at λ = 321 nm and λ = 240 nm, respectively.

3.5. Calibration curve for tiemonium methylsulphate at 235 nm

The calibration curves constructed according to the general procedure by plotting absorbance taken at 235 nm against corresponding concentrations for five standard solutions containing 1-10 µg/mL of tiemonium methylsulphate. The calibration curves are shown in Figure 11 for TMS determination. The line plot between the absorbance and the amount TMS was drawn and the straight line obeyed the equation y = 0.0318x + 0.0125 for TMS, having regression coefficient of r^2 0.9991.

3.6. Calibration curve for KTR in mixture of drugs at 320 nm

The calibration curve was made as described in the experimental procedure for KTR and correlation coefficient for maximum absorbance at 320 was found to be 0.9996. It was constructed by plotting absorbance against corresponding concentrations for five standard solutions containing 1-10 µg/mL of ketorolactromethamine. The calibration curves of mixture solution are shown in Figure 12 for TMS and KTR determination. The linearity range, regression equation and correlation coefficient were obtained by the method of least squares. The calibration equation y = 0.064x + 0.0187 was obtained for KTR determination at peak 320 nm.

3.7. Estimation TMS in mixture from the calibration equation

Calibration equation for KTR at 320 nm stand, y_K = 0.0638x_K + 0.0155 (1a) and at 245 nm, y_2 = 0.0272x_K + 0.0022 (2a) for TMS at 245 nm, y_T = 0.0318x_T + 0.0125 (3a) where, c_1 = 0.0155, c_2 = 0.0022, c_3 = 0.0125, m_1 = 0.0638, m_2 = 0.0272, m_3 = 0.0318.

Putting these values in Equation (16) constants are obtained as,

$$k_1 = \frac{1}{m_3} = 31.45 \quad (17)$$

$$k_2 = \frac{m_2}{m_3 m_1} = 13.33 \quad (18)$$

$$k_3 = \frac{m_2 c_2}{m_3 m_1} - \frac{c_2}{m_3} - \frac{c_3}{m_3} = \frac{m_2 c_2 - m_1 c_2 - m_1 c_3}{m_3 m_1} \quad (19)$$

$$k_3 = \frac{(0.0005086 - 0.000141 - 0.008)}{0.00204} = -0.21196 \quad (20)$$

Therefore, Equation (16) stands for,

$$x_T = 31.45 y_{K+T} - 13.33 y_K - 0.21196 \quad (21)$$

TMS might be determined using as Equation (21), where x_T = Concentration of TMS at 240 nm, $y_{(K+T)}$ = Total absorbance of KTR and TMS at 240 nm, and y_K = Absorbance of KTR at 320 nm. The concentration of TMS in mixture was calculated from the above equation by knowing the absorbance of KTR and total absorbance at 240 nm for KTR and TMS in mixture. Intraday and inter-day analytical data has been tabulated in Table 1.

Table 1. Summarizes intraday and inter-day precision and accuracy data for KTR and TMS.

Analysis	Added	KTR			TMS		
	Conc. (µg/mL)	Found Conc. (n=5) (µg/mL)	RSD (%)	Recovery (%)	Found Conc. (n=5)	RSD (%)	Recovery (%)
Intra day	1	1.01±0.02	1.97	101.09	0.99±0.02	2.02	99.06
	3	2.97±0.04	1.35	99.01	3.01±0.04	1.33	100.11
	5	5.11±0.04	0.78	102.04	5.01±0.04	0.79	100.31
	7	7.06±0.07	0.99	100.87	6.90±0.07	1.01	98.61
	10	9.95±0.03	0.26	99.45	10.08±0.03	0.26	100.78
Inter day	1	1.01±0.02	2.08	101.09	0.03±0.04	0.96	95.91
	3	2.89±0.03	1.21	96.39	0.08±0.10	2.88	95.91
	5	5.10±0.05	0.98	102.04	0.14±0.16	4.89	97.79
	7	7.06±0.06	0.91	100.87	0.19±0.23	6.78	96.81
	10	9.94±0.06	0.65	99.45	0.28±0.32	9.67	96.69

Table 2. Determination of KTR in alone and from mixture by calibration equation.

Standard KTR at 320 nm, y=0.0638x+0.0155				Standard KTR in mixture at 320 nm, y = 0.064x + 0.0187		
Conc. added	Conc. found (n=5)	RSD (%)	Recovery (%)	Conc. found (n=5)	RSD (%)	Recovery (%)
1	1.01±0.02	1.97	101.09	1.00±0.02	1.99	100.46
3	2.97±0.04	1.34	99.00	3.06±0.04	1.30	102.23
5	5.10±0.04	0.78	102.03	5.02±0.06	1.19	100.40
7	7.06±0.07	0.99	100.87	6.78±0.05	0.72	98.50
10	9.94±0.026	0.26	99.45	10.05±0.09	0.89	100.51

Table 3. Validation parameters of the proposed UV Spectrophotometric method.

Validation parameters	KTR	TMS
Measurement wavelength (nm)	320	235
Linear range (µg/mL)	0.8-10	1-10
Linearity equation	y=0.0638x+0.0155	y=0.0318x+0.0125
Standard deviation of the slope	0.004	0.004
Correlation coefficient (r)	0.9995	0.9991
Relative standard deviation (% RSD)		
Intraday	0.26-1.97	0.26-2.02
Inter day	2.8-5.5	0.96-9.67
Relative standard deviation (% R)		
Intraday	99.01-102.04	98.61-100.78
Inter day	96.39-102.04	95.91-97.79
Limit of detection, LOD (µg/mL)	0.25	0.31
Limit of quantification, (µg/mL)	0.80	0.95

3.8. Method validation

3.8.1. Sensitivity

The limit of detection were calculated from calibration graph by the formula; LOD = $3\times S_{xy}/a$, and the limit of quantification; LOQ = $10\times S_{xy}/a$. The LOD of KTR and TMS were found to be 0.40 and 0.50 µg/mL, respectively, and the LOQ of KTR and TMS were found to be 1.330 and 0.082 µg/mL. These results indicate that method is sensitive enough for therapeutic assay.

3.8.2. Recovery and accuracy

The results of recovery studies obtained from the intra-day assay at five concentrations (n = 5) by the proposed method in the range 99.01-102.04% for KTR, 98.61-100.78% for TMS and for inter-day assay the corresponding values in the range 96.39-102.04% for KTR, 95.91-97.79% for TMS indicating the high Accuracy of the drug. Intra-day and inter-day recovery data for proposed method are presented in Table 1.

3.8.3. Precision

The relative standard deviations (RSD) obtained for the intraday assay at five concentrations (n = 5) in the range 0.26-1.97% for KTR and 0.26-2.02% for TMS and for inter-day assay the corresponding values in the range 0.65-2.08% for KTR and 0.96-9.67% for TMS indicating the high precision of the method. Intra day and inter-day precision data for proposed method are presented in Table 1.

3.8.4. Robustness

The concentration of KTR were calculated from the calibration equation y = 0.0638x + 0.0155 at 320 nm and that of KTR in mixture were calculated from the calibration equation y = 0.064x + 0.0187 at 320 nm. Both results were compared as given in Table 2. Both the case estimation of KTR has been performed with comparable recovery and precision. So, the concentration of KTR in mixture remained constant. Above data proved that there is no drug interaction in their combined mixture.

3.8.5. 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 of the sample at 5 µg/mL concentration level including within- and between-day assessments for standard. Precision of found concentration and Relative standard deviations were examined to evaluate the system suitability. Relative standard deviations (RSD) were 0.809 and 2.510% for KTR and mixture of (KTR+TMS) respectively indicates the excellent suitability for the proposed method. Validation performances of the proposed UV Spectrophotometric method are presented in Table 3.

3.8.6. Stability

The stability of KTR and TMS in methanol, stored in clear glassware in the fridge (4 °C) was tested at five intervals by the 30 days. The responses from the aged solutions were compared with those from freshly prepared standard solution.

Table 4. Determination of ketorolactromethamine in pharmaceutical formulation by the proposed UV spectrophotometric method.

Drug name	Brand name	Conc. (µg/mL)		R (%)	% RSD	Aver. R (%)	% R by UPLC	Variation (%)
		Added	Found					
Tablet								
Etorac	Incepta	1.00	0.75	74.45	2.94	73.47	75.33	2.46
		3.00	2.25	74.97				
		5.00	3.55	71.00				
Ketonic	Sk+f	1.00	0.62	61.91	2.63	61.14	61.96	1.32
		3.00	1.79	59.29				
		5.00	3.11	62.22				
Rolac	Renata	1.00	0.67	66.61	3.00	68.84	66.15	-4.06
		3.00	2.08	69.23				
		5.00	3.53	70.69				
Xidolac	Beximco	1.00	0.84	83.86	4.06	83.67	85.13	1.72
		3.00	2.41	80.19				
		5.00	4.35	86.99				
Torax	Square	1.00	0.67	66.61	2.30	68.18	67.40	-1.16
		3.00	2.06	68.18				
		5.00	3.49	69.75				
Injection								
Torax	Square	3.00	2.25	74.97	0.69	75.34	75.95	0.80
		5.00	3.79	75.70				
Rolac	Renata	3.00	1.59	55.03	2.75	55.12	54.34	-1.43
		5.00	2.86	57.21				

Table 5. Determination of tiemonium methylsulphate in pharmaceutical formulation by the proposed UV spectrophotometric method.

Drug name	Brand name	Conc. (µg/mL)		R (%)	Avr. R (%)	RSD (%)	% R by UPLC	Variation (%)
		Added	Found					
Tablet								
Visrul	Opsonin	3.00	2.31	77.04	77.98	1.71	76.06	-2.52
		5.00	3.94	78.93				
Norvis	Square	3.00	2.68	89.62	88.36	2.01	86.41	-2.25
		5.00	4.35	87.11				
Algin	Renata	3.00	2.18	72.85	70.55	3.59	72.56	2.77
		5.00	3.41	68.24				
Timozin	Incepta	3.00	2.25	74.95	72.85	4.07	73.88	1.39
		5.00	3.54	70.75				
Visceralgin	Nurvista	3.00	2.25	74.95	75.99	1.94	76.06	0.09
		5.00	3.85	77.04				
Injection								
Algin	Renata	3.00	2.18	72.85	71.17	3.34	71.13	-0.06
		5.00	3.47	69.49				
Visceralgin	Nurvista	3.00	2.31	77.04	77.04	0.92	69.63	-10.64
		5.00	3.85	76.04				
Syrup								
Algin	Renata	3.00	2.22	73.89	75.15	2.37	75.44	0.38
		5.00	3.82	76.41				
Viseralgin	Nurvista	3.00	2.03	67.61	67.92	0.64	69.25	1.92
		5.00	3.41	68.23				

The results showed that the absorbance of KTR remained almost unchanged and no significant degradation within the indicated period occurred. Recovery of KTR was ≥ 95 % up to 30 days and TMS was ≥ 85 % up to 15 days at 4 °C stored sample as shown in Figure 13. Results conclude that there was no degradation product and KTR is stable at 4 °C for at least 30 days and TMS is stable at 4 °C for at least 15 days, indicating the possibility of using the samples over a period of 30 days and 15 days for KTR and TMS respectively at refrigerator without degradation.

4. Applications

4.1. Pharmaceutical formulations

The method developed here was applied to three concentrations (1.0, 3.0 and 5.0 µg/mL) of solutions prepared from pharmaceutical products for determining the content of KTR and TMS. When KTR was determined using equation 1a and TMS was determined by Equation (21). The values of the overall drug percentage recoveries and the RSD values of measurements are as presented in Tables 4 and 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.

4.2. Application to bio-samples

The method developed here was applied to various spiked concentration of solutions prepared from biological samples 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 Tables 6 and 7. Results indicate that measurements are acceptable with good precision.

5. Conclusion

The spectrophotometric method developed herein is simple, inexpensive and sensitive enough for estimation of both drugs. TMS was estimated without having its absorbance recorded. TMS not only estimated in mixture but also its LOD was increased due to additives properties. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. Results were comparable to that of UPLC method [16].

Table 6. Determination of ketorolac tromethamine in biological samples by the proposed UV spectrophotometric method.

Bio samples	Drug injection	Conc. (µg/mL)		Blood conc.	RSD (%)	R (%)	R (%) (UPLC)	Variation (%)
		Spiked	Found					
Blood	Rolac	0.00	0.92	0.92	2.16	-	-	-
		1.00	1.90	0.91	1.57	97.79	108.4	9.81
		3.00	3.93	0.93	0.76	100.2	99.97	-0.19
Urine	Rolac	0.00	0.56		0.89	-	-	-
		1.00	1.56	0.56	1.28	100.6	93.10	-8.01
		3.00	3.56	0.57	0.84	101.7	95.28	-6.72

Table 7. Determination of tiemonium methylsulphate in biological samples by the proposed UV spectrophotometric method.

Bio samples	Drug injection	Conc. (µg/mL)		Blood conc.	RSD (%)	R (%)	R (%) (UPLC) [16]	Variation (%)
		Spiked	Found					
Blood	Align	0.00	0.833		1.2	-	-	-
		1.00	1.839	0.839	1.6	100.75	102.2	1.42
		3.00	3.821	0.821	0.8	98.49	101.9	3.35
Urine	Align	0.00	0.060		1.6	-	-	-
		1.00	1.059	0.059	1.9	97.44	99.8	2.55
		3.00	3.060	0.060	1.0	98.46	98.4	-0.06

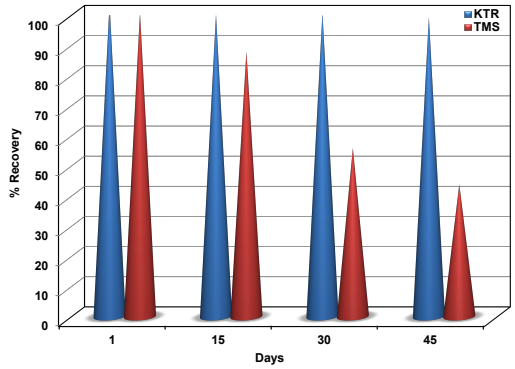

Figure 13. Long stability graph for KTR and TMS.

Method was successfully validated as per ICH guidelines. It can be conveniently employed for routine quality control analysis of ketorolac tromithamine and tiemonium methylsulphate in pharma-ceutical formulation and biological samples without any interference. Attempt might be taken to develop software based on Equation (21) for the estimation of TMS.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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