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Validated Spectrophotometric Methods for the Determination of Ketorolac Tromethamine

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Summary: Three simple and accurate spectrophotometric methods were developed for the deternmination of ketorolac-tromethamine. The first one was based on the reaction of carboxylic acid group of drug with a mixture of KIO_3/ KI to form yellow free I_2 in aqueous medium exhibiting λ_{max} at 350 nm. The second method was depended on the reaction of the drug as an n-donor with two π-acceptors; 2,6-dichlorophenol indophenol and 2,3-dichloro-1,4-naphthaquinone to yield highly colored radical anions in the polar solvents used having λ_{max} 645 nm and 492 nm, respectively. The third method used the reducing characters of tromethamine moeity of drug to produce an orange-red colored ferroin complex with λ_{max} 514 nm from ferriin and red formazan from triphenyltetrazolium chloride and subsequent measurement at 484 nm. Beer·s law was found to be obeyed for the cited drug at the concentration ranges of 5-25, 5-70, 30-270, 5-25 and 40-240 µg mL^{-1} ketorolac-TM for the five reagents used, respectively. The proposed methods have been successfully applied to analyze the studied drug in pharmaceutical formulations with mean recoveries ranged from 97.52%±0.36 to 101.54%±1.58. The methods were validated according to ICH guidlines.

Introduction

Ketorolac-tromethamine (ketorolac-TM) is (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid with 2-amino-2-(hydroxymethyl)-1,3-propanediol[(1)]. It is a NSAID, much more potent than aspirin, exhibiting pronounced analgesic and moderate anti-inflammatory activity. It is indicated for the short term management of moderate to severe painful states such as post operative pain, acute musculoskeletal pain and dental pain[(1)]. Reviewing the literature, several analytical techniques have been published for its estimation in pharmaceutical formulations and in biological fluids including HPTLC[(2)], GC-MS[(3)], LC[(4)], HPLC[(5,6)] and spectrophotometry[(7-9)].

The main target of this work is to develop simple and efficient spectrophotometric methods for the determination of the drug in bulk powder and pharmaceutical formulations.

Experimental

Apparatus

Shimadzu UV/Vis spectrophotometer PC – 1601, (Tokyo, Japan), Jenco pH meter (608) with jenway double junction glass electrode, (England) and water bath (SONAMAK, UTA-60, Italy) were used in this investigation.

Samples

Pure Ketorolac-TM, B.N. (KL050010), was kindly supplied by Amriya Pharmaceutical Company, Egypt with purity of 99.16% according to the supplier, Ketolac® tablets, B.N. (505325), labelled to contain 10 mg Ketorolac -TM per tablet, Ketolac® ampoules, B.N. (463516A), each 1 mL contains 15 mg Ketorolac -TM, products of Amriya Pharmaceutical Company, Egypt, were purchased from local market and Acular® eye drops, B.N. E65071, labelled to contain 0.5% of Ketorolac -TM, a product of Allergan Pharmaceutical Company, Ireland, were purchased from local market.

Chemicals and reagents

All chemicals and reagents used were of analytical grade and solvents were of spectroscopic grade.

Methanol, absolute ethanol, acetonitrile and chloroform were obtained from Sigma – Aldrich, USA. Dimethylformamide, 1,4 dioxane and HCl were Riedell-detlean, Germany products. Glacial acetic acid, sodium acetate (anhydrous), NaOH and anhydrous Na_2SO_4 were obtained from EL-Nasr Co., Egypt. Ammonium iron (III) sulfate, $NH_4Fe(SO_4)_2.12H_2O$ was Winlab, U.K. product and 1,10 Phenanthroline was obtained from Merck, Germany.

KI aqueous solution (0.15 and 1 x 10^{-2} mol L^{-1}) were freshly prepared by dissolving 2.49 g and 0.166 g, respectively in 100 mL distilled water. KIO_3 (Oxford, Mumbai, India) freshly prepared aqueous solutions (0.1 and 1 x 10^{-3} mol L^{-1}) were prepared by dissolving 2.14g and 0.0214 g, respectively in 100 mL distilled water. 2,6-Dichlorophenol indophenol, DCPI (Winlab, France), 0.1% and 1x10^{-3} mol L^{-1} solutions in chloroform. They were prepared by dissolving about 0.1gm or 0.027g in 30 mL water, transferred to separators then rendered acidic with 5 mL 2 N HCl and extracted with four successive 20 mL portions of chloroform. The chloroform

extracts were pooled through anhydrous Na_2SO_4 into a 100 mL volumetric flask and diluted to volume with chloroform[10]. 2,3-Dichloro-1,4-naphthaquinone, Dichlone (Fluka biochemika, Switzerland), 0.9% and 1x10^{-2} mol L^{-1} solutions prepared by dissolving 0.9 g and 0.23 g in 100mL DMF. Acetate buffer solutions (pH 3-5.8), were prepared by dissolving 10 g of sodium acetate anhydrous in 300 ml water, adjusting the pH with glacial acetic acid and diluting to one liter with water[11]. Phen-Fe(III) mixture[12], was prepared by dissolving 0.5 g of o-phenanthroline monohydrate and 0.4 g ammonium ferric sulfate in 5 mL 1 N hydrochloric acid, then diluted to 250 mL with water. This solution is stable for one month in refrigerator. Also 7.5 x10^{-3} mol L^{-1} of this reagent was prepared by dissolving about 0.15 g of o-phenanthroline monohydrate and 0.12 g ammonium ferric sulfate in 5 mL 1N hydrochloric acid then diluted to 100 mL with water. Alcoholic NaOH 0.2 N solution[12], was prepared by dissolving 0.8 g in minimum amount of water and completing to 100mL with ethanol. 2,3,5 Triphenyltetrazolium chloride (tetrazolium red, Reidel, Germany); 0.2% and 1 x10^{-2} mol L^{-1} were prepared by dissolving about 0.2 g and 0.335 g in 100 mL absolute alcohol.

Standard solutions

0.25 mg mL^{-1}, 1 x 10^{-3} and 0.75 x 10^{-2} mol L^{-1} drug solutions were prepared by dissolving 0.025, 0.038 and 0.282 g ketorolac-TM in 100 mL water, respectively.

0.5 mg mL^{-1} and 1 x10^{-3} mol L^{-1} drug solutions were prepared by dissolving 0.05 and 0.038 g ketorolac-TM in 100 mL in methanol, respectively.

1 mg mL^{-1} and 1x10^{-2} mol L^{-1} drug solutions were prepared by dissolving 0.1 g and 0.376 g ketorolac-TM in 100 mL of DMF, respectively.

1 mg mL^{-1} and 1x10^{-2} mol L^{-1} drug solutions were prepared by dissolving 0.1 g and 0.376 g ketorolac-TM in 100 mL absolute ethanol, respectively.

Procedures

Iodate-iodide method

Into a series of 20-mL test tubes, aliquots of standard ketorolac-TM solution (0.25 mg mL^{-1}) equivalent to 0.05- 0.25 mg were introduced. Then 2 mL of 0.1 mol L^{-1} KIO_3 were added followed by 3 mL of 0.15 mol L^{-1} KI. The tubes were mixed well, covered and heated in water bath for 10 min at 50°C then cooled and transferred quantitatively

into series of 10-mL volumetric flasks. Volume was then diluted with water and absorbance of the developed yellow color was measured at wavelength 350 nm against a similarly prepared blank.

Charge transfer complexation

DCPI - Aliquot volumes of standard methanolic drug solution (0.5 mg mL^{-1}) containing 0.05-0.7 mg of ketorolac-TM were transferred into a series of 10 mL volumetric flasks. Three mL of 0.1% DCPI solution in $CHCl_3$ were added. The volumes were adjusted with methanol and the absorbance of the bluish violet colour was measured at λ_{max} 645 nm against a reagent blank.

Dichlone - Into a series of 20 mL test tubes, aliquots of standard DMF drug solution (1 mg mL^{-1}) containing 0.3-2.7 mg of Ketorolac-TM were introduced. Then 1 mL of 0.9% Dichlone solution in DMF was added and volume completed to 8 mL with DMF. The tubes were mixed well and heated at at 100°C for 30 min, cooled and transferred into a series of 10 mL volumetric flasks. Volumes were adjusted with DMF and the absorbance of the orange colour was measured at 492 nm against a reagent blank.

Redox method

Phen-Fe(III) - Into a series of 20-mL test tubes, aliquots of standard aqueous ketorolac-TM solution (0.25 mg mL^{-1}) equivalent to 0.05-0.25 mg mL^{-1} were introduced. Then 1.5 mL of Phen-Fe(III) mixture and 1 mL of acetate buffer of pH 4.5 were added to each tube. Volume completed to 8 mL with water then heated at 100 °C for 30 min, cooled and transferred into a series of 10 mL volumetric flasks. Volumes were adjusted with water and the absorbance of the developed orange red color was measured at 514 nm against a reagent blank.

Tetrazolium red (TZR) - Into a series of 20-mL test tubes, aliquots of standard ketorolac-TM solution (1 mg mL^{-1}) equivalent to 0.4-2.4 mg were introduced. 0.5 mL of aqueous tetrazolium red solution and 0.5 mL of alcoholic sodium hydroxide were added to each tube and completed to 4mL with ethanol. The tubes were mixed well, covered, heated at 60°C for 20 min, cooled and transferred quantitatively into a series of 10-mL volumetric flasks. Volume was then completed with ethanol and the absorbance of the developed red color was measured at 484 nm against a reagent blank.

Application to pharmaceutical formulations

A- Ketolac® Tablets- Forty tablets were weighed and finely ground. An amount of fine powder equivalent to 25 mg, 50 mg and 100 mg of ketorolac-TM were weighed in a 100- mL volumetric flasks and extracted by shaking with 70 mL water, methanol, DMF and absolute ethanol, respectively for about 10 min. Then the volume was completed to the mark with the previous solvents, respectively. The clear filtrate labeled to contain 0.25 mg mL^{-1}, 0.5 mg $mL^{-1}$and 1 mg $mL^{-1}$of ketorolac-TM in water, methanol, DMF and absolute ethanol were analyzed using IO_3^- / I^- solution, DCPI, Dichlone, Phen-Fe(III) and TZR, respectively as detailed under "Procedure".

B- Ketolac® Ampoules- The solution of twelve ketolac ampoules were mixed well, a portions of the mixed solution equivalent to 30 mg, 60 mg and 105 mg of ketorolac-TM were introduced into a 100-mL volumetric flasks and diluted to the volume with water, methanol, DMF and absolute ethanol. The solution labeled to contain 0.3 mg mL^{-1}, 0.6 mg mL^{-1} and 1.05 mg mL^{-1} of ketorolac-TM in water, methanol, DMF and absolute ethanol were analyzed using the five reagents respectively as detailed under "Procedure".

C-Acular® eye drops- The solution of ten Acular drops containers were mixed well, portions of the mixed solution equivalent to 25 mg, 50 mg and 100 mg of ketorolac-TM were introduced into a 100-mL volumetric flasks and dilute to the volume with water, methanol, DMF and absolute ethanol. The solutions labeled to contain 0.25 mg mL^{-1}, 0.5 mg mL^{-1} and 1 mg mL^{-1} of ketorolac-TM in water, methanol, DMF, water and absolute ethanol, respectively were analysed using the five reagents as detailed under "Procedure".

Results and discussion

Iodate-iodide method

It has been reported that water-soluble organic acidic compounds may liberate iodine from a solution containing both KIO_3 and KI according to the reaction[13]:

$$5\ I^- + IO_3^- + 6\ H^+ \rightarrow 3\ H_2O + 3\ I_2$$

The yellow colour obtained is due to the formation of I_2, immediately converted into triiodide ions exhibiting an absorption maxima at 298 and 360 nm[13]. The confirmatory test for the presence of iodine in the final solution of the drug was

established by the blue color with starch solution. Ketorolac-TM, containing a carboxylic group was suspected to liberate I_2 from IO_3^-/I^- mixture which was found to have two λ_{max} 288 nm and 350 nm; (Fig.1). The peak due to yellow colour of aqueous I_2 at 350 nm was used for the spectrophotometric determination of drug.

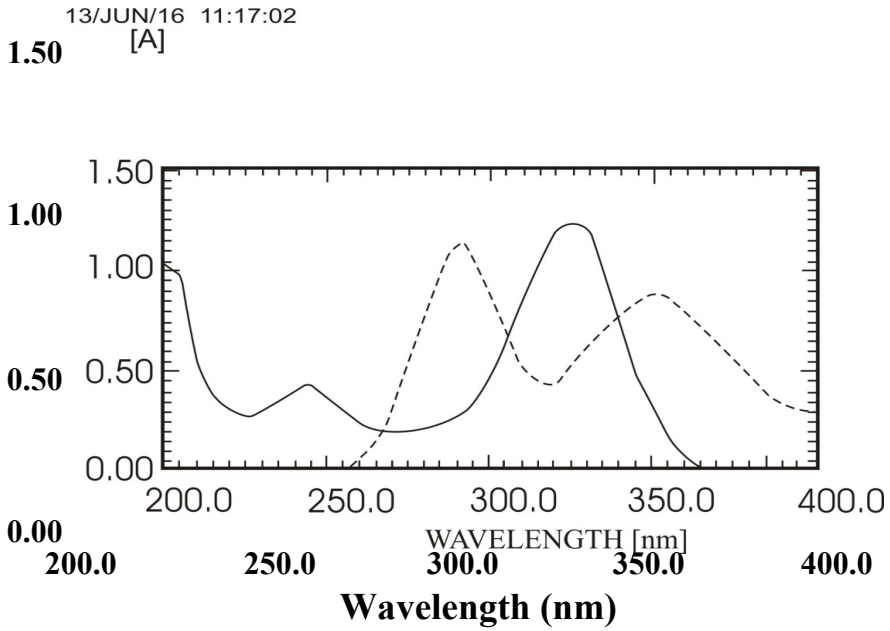
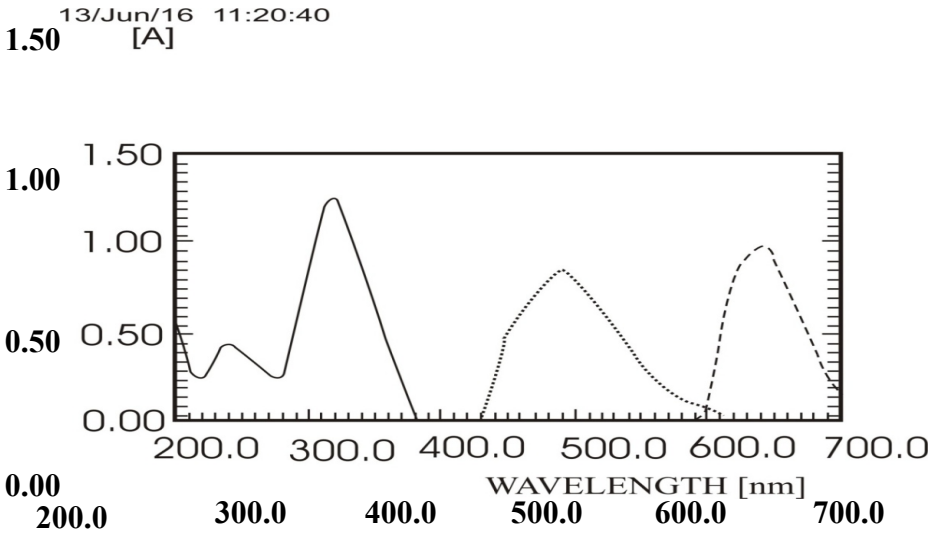


Figure (1): Absorption spectra of 20 µg mL^{-1} ketorolac-TM in water (−) and its reaction product with KIO_3/KI in water (---).

Charge-transfer complxation method

Ketorolac-TM as a nitrogenous compound was expected to act as an n-donor for some π-acceptors. Upon reacting it with DCPI in 3:7 $CHCl_3$-methanol or with Dichlone in DMF, charge-transfer complexes were obtained. Then complete charge transfer from donor to acceptor was facilitated by polar solvents used to give bluish violet or orange radical anions absorbing maximally at 645 or 492 nm, respectively; (Fig. 2).

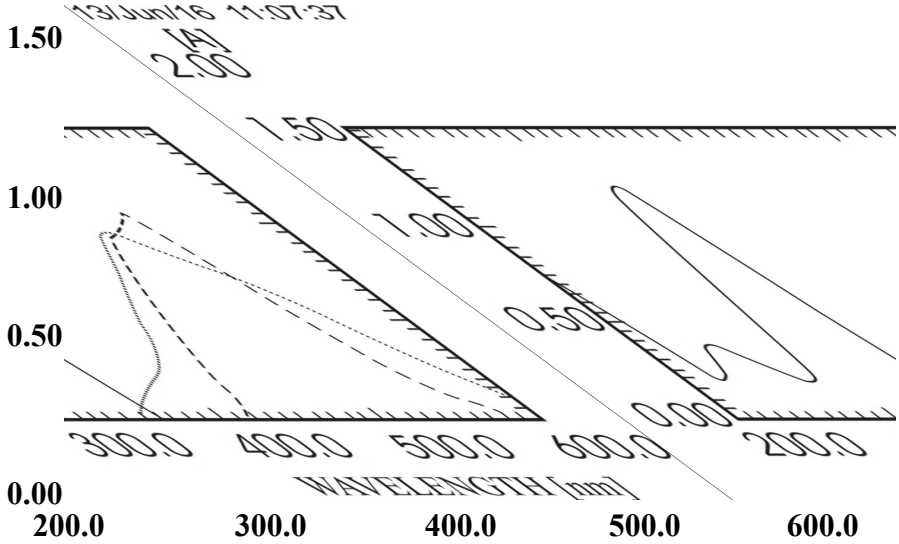


Wavelength (nm)

Figure (2): Absorption spectra of 20 µg mL^{-1} ketorolac-TM in methanol (−), its reaction product with DCPI in $CHCl_3$-methanol (3:7) (---) and with dichlone in DMF (….).

Redox method

This method was based on the reducing character of tromethamine moeity of Ketorolac-TM on Fe(III) to Fe(II) in its complex with 1,10- phenanthroline to give the orange-red colored ferroin complex with λ_{max} 514 nm or on the reduction of TZR to red formazan absorbing maximally at 484 nm; (Fig. 3).



Wavelength (nm)

Figure (3): Absorption spectra of 20 µg mL^{-1} ketorolac-TM in absolute ethanol (−), its reaction product with phen-Fe (III) in water (---) and with tetrazolium red in alkaline ethanol (….).

Optimization of experimental parameters

Effect of reagent volume

Different volumes of 0.1 mol L^{-1} KIO_3 were allowed to react with drug in presence of excess KI. Again different volumes of 0.15 mol L^{-1} KI (0.5-4.5 mL) were studied in presence of KIO_3. The absorbance at 350 nm was found to increase gradually; reaching its maximum on using 2 mL of 0.1 mol L^{-1} KIO_3 and 3 mL of 0.15 mol L^{-1} KI and remained constant beyond these volumes. Thus 2 mL of 0.1 mol L^{-1} KIO_3 and 3 mL of 0.15 mol L^{-1} KI were recommended.

For charge transfer complexation, 2.5-3.5 mL of 0.1% DCPI in $CHCl_3$ or 0.8-1.2 mL of 0.9% dichlone were found to give maximum colour intensity at 645 nm or 492 nm, respectively. While for redox reactions, 1.2-2.0 mL of phen-Fe(III) mixture and 0.4-0.6 mL of TZR gave maximum absorbance at 514 and 484 nm, respectively.

Effect of temperature and heating time

The reaction of the drug with all the reagents used was slow at room temperature except for DCPI that reacted spontaneously at room temperature. Thus elevated temperature was used to accelerate the reaction with other reagents using water bath at 40-100°C.

KIO_3/KI required a heating time of 10 min at 50 °C with the drug while complete reaction with dichlone or phen-Fe(III) reagents was attained at 100°C after 30 min or 20 min, respectively. For TZR, heating time of 15 min at 60°C gave maximum colour and thus it was used throughout the work.

Effect of acidity or alkalinity

The drug was allowed to react with phen-Fe(III) in presence of 2 mL acetate buffer of different pH values (3.0-5.8). Where optimal pH was found to be 4.3-4.7. Volume of acetate buffer pH 4.5±0.2 was studied, where 0.8-1.2 mL was found to be sufficient to give maximum colour at 514 nm. Thus 1 mL of acetate buffer pH 4.5±0.2 was used. For TZR, the reaction proceeds only in alkaline medium[12]. Therefore, 0.2-0.8 mL of 0.2 N alcoholic NaOH was added to the reaction mixture, where 0.5 mL of 0.2 N alcoholic NaOH was found to be optimal.

Effect of diluting solvent

Ethanol, acetonitrile, $CHCl_3$, methanol, dioxane and DMF were studied. For DCPI, the reagent is only soluble in $CHCl_3$ and completing with methanol gave the highest sensitivity, thus a ratio 3:7 $CHCl_3$-methanol was recommended. While for dichlone DMF was the solvent of choice for the drug, reagent and product. Water was the optimum solvent for phen-Fe(III) while absolute ethanol was the recommended solvent for TZR[12].

Stability of coloured product- All five products were stable over a period of 60 min.

Stoichiometry of the reaction

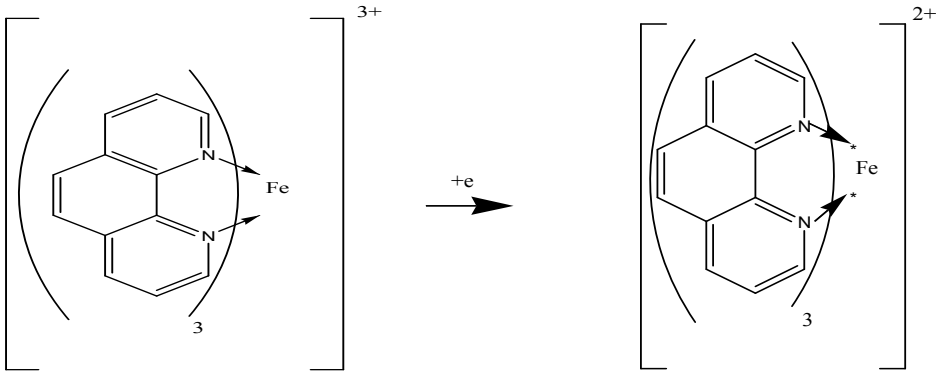
Job's method[14] was applied using ($1x10^{-3}$, $1 x10^{-2}$ and $0.75x10^{-2}$ mol L^{-1}) solutions of ketorolac-TM for KIO_3/KI, charge transfer and redox methods, respectively. 6:1 ratio between the drug and KIO_3/KI or 1:1 ratio between the drug and DCPI, Dichlone, phen-Fe(III) and TZR reagents suggesting the following mechanism:

$$6\ \text{RCOOH-TM} + IO^-_3 + 5\ I^- \xrightarrow{\text{aqueous medium}} 6\ RCOO^- + 3\ I_2 + 3\ H_2O$$

$$D + A \rightarrow D{\rightarrow}A \leftrightarrow D^{\cdot+} + A^{\bullet-}$$

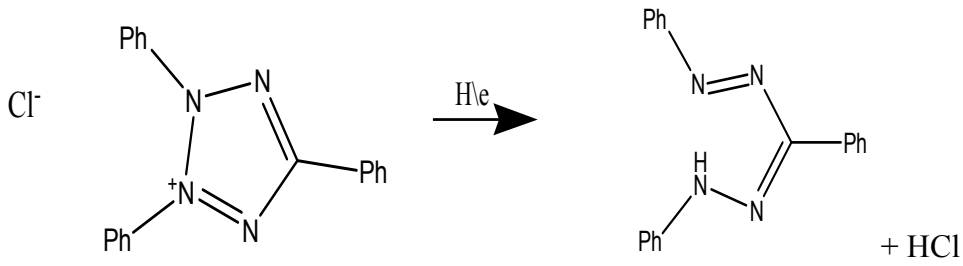
donor acceptor complex coloured radical anion

Scheme (1): The suggested reaction of Ketorolac-TM with KIO_3/KI and π-acceptors.



Ferriin Ferroin

Orange-red color



Tetrazolium Red Formazan

Scheme (2): The suggested reactions of Ketorolac-TM with phen-Fe (III) and tetrazolium red.

Validation of the methods

Linearity: Under the above optimum experimental conditions, Beer's law was obeyed in the ranges of 5-25, 5-70, 30-270, 5-25 and 40-240 µg mL^{-1} for KIO_3/KI, DCPI, Dichlone, phen-Fe(III) and TZR reagents used, respectively. The regression parameters were represented in, Table (1).

The relative sensitivities of the products using the five mentioned reagents were compared by calculating A(1%,1cm). The iodate-iodide method was found to be the most sensitive; A(1%,1cm) equals 543 at 350 nm, Table (1).

Accuracy and Precision: Three different concentrations of the drug within the same day and on three successive days were analyzed by the three proposed methods. Good accuracy was obtained that amounted to 100.11, 99.76, 99.96, 99.13 and 98.70% for the five reagents used, respectively. The intraday precision calculated as RSD ranged between 0.28 and 1.93% while the intermediate precision over a period of two weeks were from 0.48 to 1.77%; Table (1).

Robustness: It was examined by small variation in volumes of the five reagents. It was observed that no significant change in absorbance and RSD% was not greater than 1.39.

Ruggedness: Time for the reaction of the studied drug with KIO_3/KI and phen-Fe(III); results obtained from day-to-day variations (within 2 months) were reproducible and RSD% did not exceed 1.97. While for DCPI, dichlone and TZR two different sources of chloroform, DMF and ethanol were used and RSD ≤ 1.3%.

Table (1): Assay and validation parameters of the proposed spectrophotometric methods for the determination of ketorolac-TM

Parameter	IO_3^-/I^-	DCPI	Dichlone	Phen-Fe(III)	TZR
$\lambda_{max\ (nm)}$	350	645	492	514	484
Linearity range (μg ml^{-1})	5-25	5-70	30-270	5-25	40-240
A(1%,1cm)	543.7	174.3	33.3	464.8	38.9
Regression parameters					
Slope (b)	0.0546	0.0167	0.0033	0.0487	0.0039
Intercept (a)	-0.0131	0.0228	0.0111	-0.0240	0.0025
Correlation coefficient (r^2)	0.9992	0.9992	0.9989	0.9991	0.9998
Accuracy(R%)	100.11	99.76	99.96	99.13	98.70
Precision (RSD%)*					
Intraday	0.28-0.78	0.39-1.20	0.62-1.44	0.12-0.41	0.23-1.93
Interday	0.48-1.21	0.26-1.77	0.67-1.37	0.24-0.81	0.22-0.98

Ruggedness (RSD%)	1.73	1.29	1.20	1.97	1.08
Robustness (RSD%)	1.37	0.70	1.39	0.60	1.32

* Average of 9 determinations

Application to Pharmaceutical formulations

Good recoveries of ketorolac-TM from its Ketolac tablets, ampoules and acular eye drops were obtained ranging between 96.32%±1.24 and 101.54%±1.15, indicating non interfering excipients and additives. These results were statistically analyzed and found to be in accordance with those of a compendial method[7] which involved spectrophotomeric determination of ketorolac-TM by charge-transfer complexation with DDQ at 392 nm; Tables (2-4).

The validity of the proposed methods were further assessed by applying the standard addition technique where the mean% recoveries amounted to 97.68 -102.65% ± 0.63 - 1.76, Tables (2-4).

Table (2): Statistical comparison of the results obtained for analysis of ktorolac-TM in tablets by the proposed and reported methods[7] .

Parameter	Ketolac® tablets					
	IO_3^-/I^-	DCPI	Dichlone	Phen-Fe(III)	TZR	The Reported method[7]
Linearity range (µg mL^{-1})	5-25	5-70	30-270	5-25	40-240	10-80
N	5	5	5	5	5	5
Mean %	100.21	101.54	101.34	99.63	100.39	100.77
S.D.	1.14	1.15	1.58	0.89	1.30	0.90

Variance	1.30	1.32	2.50	0.79	1.69	0.81
t-test	0.86	1.18	0.70	2.01	0.54	-
F-test	1.60	1.63	3.08	1.02	2.09	-
Standard addition Mean± RSD%	102.15±1.29	99.55±1.48	100.64±1.27	100.90±1.62	98.49±1.17	-

The theoritical t- and F- values at P=0.05 are 2.31 and 6.39 , respectively.

Table (3): Statistical comparison of the results obtained for analysis of ktorolac-TM in ampoules by the proposed and reported methods[7] .

Parameter	Ketolac® ampoules					
	IO_3^-/I^-	DCPI	Dichlone	Phen-Fe(III)	TZR	The Reported method[7]
Linearity range (μg mL^{-1})	6-24	6-66	42-252	6-24	42-231	12-78
N	5	5	5	5	5	5
Mean %	100.38	99.87	99.48	99.35	100.05	99.84
S.D.	1.10	0.36	0.56	1.07	1.09	0.47
Variance	1.21	0.13	0.31	1.14	1.19	0.22
t-test	1.00	0.11	1.09	0.93	0.40	-

F-test	5.48	1.70	1.42	5.18	5.38	-
Standard addition Mean± RSD%	102.65±1.52	97.68±1.43	100.94±1.10	100.57±1.61	100.85±1.20	-

The theoritical t- and F- values at P=0.05 are 2.31 and 6.39 , respectively.

Table (4): Statistical comparison of the results obtained for analysis of ktorolac-TM in eye drops by the proposed and reported methods[7] .

Parameter	Acular® eye drops					
	IO_3^-/I^-	DCPI	Dichlone	Phen-Fe(III)	TZR	The Reported method[7]
Linearity range (μg mL^{-1})	5-25	5-70	30-270	5-25	40-240	10-80
N	5	5	5	5	5	5
Mean %	96.32	97.62	97.66	97.92	97.52	96.95
S.D.	1.24	1.13	0.73	0.95	1.13	0.94
Variance	1.54	1.28	0.53	0.90	1.28	0.88
t-test	0.91	1.02	1.34	1.61	0.87	-

F-test	1.74	1.45	1.66	1.02	1.45	-
Standard addition Mean± RSD%	97.99±1.69	97.91±1.35	101.49±0.63	99.74±1.76	98.12±0.96	-

The theoritical t- and F- values at P=0.05 are 2.31 and 6.39 , respectively.

Conclusion

The demand for simple, accurate and precise methods for the determination of ketorolac-TM was achieved throughout this work by the development of spectrophotometric methods through liberation of I_2 from KIO_3/KI, charge transfer complexation with DCPI and Dichlon or redox reaction with phen-Fe(III) and tetrazolium red. The simplicity of the assays could allow for their use in routine and quality control analysis.

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