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Q- Absorbance Ratio Method for Simultaneous Estimation of Ketorolac tromethamine and Phenylephrine Hydrochloride in Pharmaceutical Dosage form

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ABSTRACT

The present work describes Q- absorbance spectrophotometric method for simultaneous estimation of Ketorolac Tromethamine and Phenylephrine Hydrochloride in pharmaceutical dosage form. It employs development of Q-absorbance ratio method using two wavelengths 273 nm of Phenylephrine Hydrochloride and 284 nm an Iso-absorptive point of both the drug. The method obeys beer's law in the employed concentration range of 3-21 µg/ml for Ketorolac Tromethamine and 10-70 µg/ml for Phenylephrine Hydrochloride at their respective wavelengths. Results of analysis were validated statistically and by recovery studies as per ICH guideline. The method was found to be simple, precise, reproducible, rapid & economical.

***Key Words**: Ketorolac Tromethamine (KETO), Phenylephrine Hydrochloride (PHE), Spectrophotometric method.*

INTRODUCRTION

Phenylephrine hydrochloride is chemically (-) m-hydroxy-α-[(methylamino) methyl]benzylalcohol hydrochloride (Figure 1). Phenylephrine HCl is a α-1 adrenergic receptor agonist used for dilation of the pupil due to its vasoconstrictor and mydriatic action. Phenylephrine possesses predominantly α-adrenergic effects. In the eye, Phenylephrine acts locally as a potent vasoconstrictor and mydriatic, by constricting ophthalmic blood vessels and the radial muscle of the iris. [2, 3]

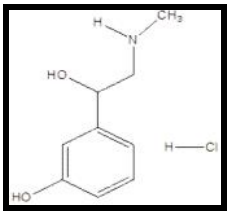

Fig. 1: Structure of Phenylephrine hydrochloride

Ketorolac tromethamine is chemically 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid - 2-amino-2-(hydroxymethyl)-1,3-propanediol. Ketorolac tromethamine is a non-steroidal anti inflammatory drug which, when administered systemically, has demonstrated analgesic, anti-inflammatory, and anti-pyretic activity. Its ability to inhibit prostaglandin biosynthesis [1, 3].

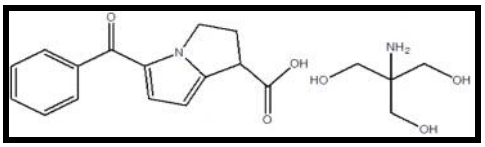

Fig. 2: Structure of Ketorolac tromethamine

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The combination of the anti-inflammatory agent Ketorolac, and the mydriatic (pupil-dilating) agent Phenylephrine. It is used during ophthalmic procedures such as cataract surgery or intraocular lens replacement (ILR) to maintain pupil size by preventing intraoperative miosis (pupil constriction) and to reduce postoperative pain.

Several spectrophotometric and chromatographic methods have been reported for the estimation of Phenylephrine and Ketorolac alone as well as with other drugs. Since no spectrophotometric method is reported for simultaneous estimation of Phenylephrine and Ketorolac in combined dosage form. Therefore, in the present work; a successful attempt has been made to estimate both these drugs simultaneously by Q-ABSORBANCE RATIO method.

MATERIALS AND METHOD

Appratus:

SHIMADZU double beam UV–visible spectrophotometer with 10mm matched quartz cell model UV 1800 (Japan) was used for the development of proposed method.

Reagents and Materials:

Phenylephrine hydrochloride (PHE) drug sample was kindly gifted from mamata pharmaceutical Waghodiya (Gujarat, India) and Ketorolac tromethamine (KETO) drug sample was kindly gifted from Cadila Pharmaceutical pvt Ltd. Dholka (Gujarat, India).

Method Development:

Solubility test for PHE and KETO was performed by using various solvents. Both drugs were freely soluble in distilled water. Hence distilled water was selected as solvent for the proposed method.

Preparation of standard stock solution of PHE: Accurately weighed quantity of PHE 100 mg was transferred into 100 ml (1000 µg/ml) volumetric flask, dissolved and diluted up to mark with distilled water and used as working standard solution.

Preparation of standard stock solution of KETO: Accurately weighed quantity of KETO 100 mg was transferred into 100 ml (1000 µg/ml) volumetric flask, dissolved and diluted up to mark with distilled water and used as working standard solution.

Preparation of working standard solution of PHE: 100 µg/ml of PHE solution was prepared by diluting 10 ml of stock solution in 100 ml with distilled water.

Preparation of working standard solution of KETO: 100 µg/ml of KETO solution was prepared by diluting 10 ml of stock solution in 100 ml with distilled water.

Preparation of Calibration Curve:

From the working standard solutions of PHE (1, 2, 3, 4, 5, 6, and 7 ml) and standard solutions of KETO (0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1 ml) was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with distilled water and mixed. The absorbance of the solutions was measured at 273 nm and 284 nm against distilled water as a blank.

Development of Q-Absorbance Ratio Method:

Q method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λmax of one of the two compounds. From the stock solutions, working standard solutions of KETO (100 µg/ml) and PHE (100 µg/ml) were prepared. By appropriate dilutions, the solutions with concentrations 3-21 µg/ml (for KETO) and 10-70 µg/ml (for PHE) were prepared and scanned between 200 to 400 nm. Series of standard solutions (for both KETO and PHE) were prepared and the absorbance of solutions was recorded at 273 nm (λmax of PHE) and 284 nm (isoabsorptive point) to plot a calibration curve of absorbance versus concentration. Calibration curves were found to be linear in the concentration range under study. Absorptivity values of KETO and PHE were determined at selected wavelengths. The concentration of two drugs in mixture was calculated by using following equations: [4, 5]

$$Cx = (Qm-Qy) \times A / (Qx-Qy) \times ax1$$
$$Cy = (Qm-Qx) \times A / (Qy-Qx) \times ay1$$

Where,

Qm=A2(Absorbance of sample at iso-absorptive point)/A1(Absorbance of sample at λmax)

Qx=Absorptivity of KETO at iso-absorptive point /Absorptivity of KETO at selected wavelength

Qy= Absorptivity of PHE at Iso-absorptive point / Absorptivity of PHE at selected wavelength

ax1= Absorptivity of KETO at Isoabsorptive point

ay1= Absorptivity of PHE at Isoabsorptive point

Validation of Methods:

Proposed methods were validated in accordance with ICH guidelines Q2 (R1) for evaluation of various parameters; linearity, limit of detection, limit of quantification, precision and accuracy.

Linearity:

Calibration curves were plotted over a concentration range of 10-70 µg/ml and 3-21 µg/ml for PHE and KETO respectively. The calibration curves were constructed by plotting absorbance *vs.* concentration.

Method Precision (Repeatability):

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of PHE and KETO (30 µg/ml and 9 µg/ml respectively) without changing the parameters for the Q-absorbance ratio method.

Intermediate Precision (Reproducibility):

The intraday and interday precisions of the proposed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days, different concentrations of standard solutions of PHE (30, 40 and 50 µg/ml) and KETO (9, 12 and 15 µg/ml). Results were reported in terms of RSD.

LOD and LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) of the drug was derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline:

The LOQ may be expressed as:

LOD = 3.3 × (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 5 calibration curves; Slope = the mean slope of the 5 calibration curves.

LOQ = 10 × (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 5 calibration curves; Slope = the mean slope of the 5 calibration curves.

Accuracy (Recovery Study):

The accuracy of the methods was determined by calculating recoveries of PHE and KETO by the standard addition method. Known amounts of standard solutions of PHE and KETO were added at 80, 100 and 120% levels to prequantified sample solutions of PHE and KETO (30 and 9 µg/ml respectively). The amounts of PHE and KETO were estimated by applying the obtained values to the Q -absorbance ratio method.

Estimation of PHE and KETO in dosage form:

Each 1 ml of dosage form containing 3 mg of KETO and 10 mg of PHE, diluted with distilled water in 100 ml volumetric flask, kept in ultrasonic water bath for 10 min to get optimum mixing of the active ingredients and diluted up to mark with distilled water (30 µg/ml of KETO and 100 µg/ml of PHE). From above solution 3 ml was taken in a 10ml volumetric flask, diluted with distilled water upto the mark (9 µg/ml of KETO and 30 µg/ml of PHE). The solution was filtered using Whatman filter paper no. 41 and first few drops of filtrate were discarded. The response of the solution was measured at 273 nm and at 284 nm. The concentration of each drug was calculated using equation of Q-absorption method. The concentration of KETO and PHE can be obtained.

RESULTS AND DISCUSSION

Q-Ratio Method:

The standard solutions of PHE and KETO were prepared separately in distilled water. They were scanned in the wavelength range of 200-400 nm. The over line spectrum of PHE and KETO, one wavelength was selected for the estimation of both drugs, and which known as iso-absorptive point (at 284 nm) is shown in Figure 3. The dilutions of standard and sample solutions were prepared. The Absorptive values were determined at 273 nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using the formula (Graph 1-4).

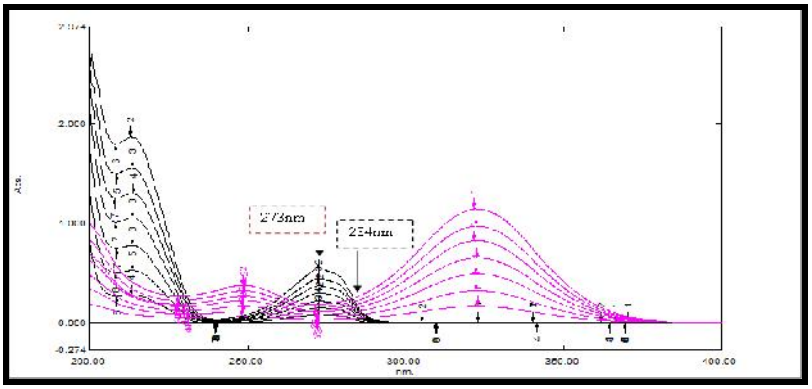
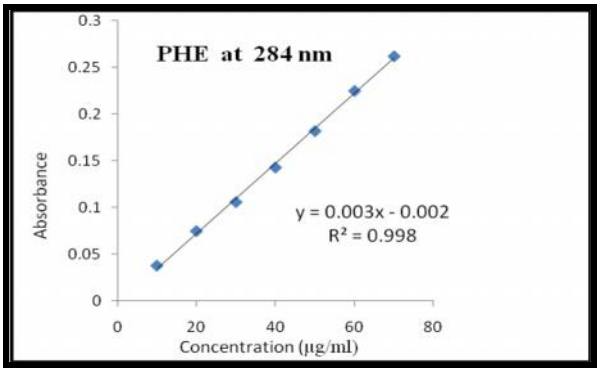
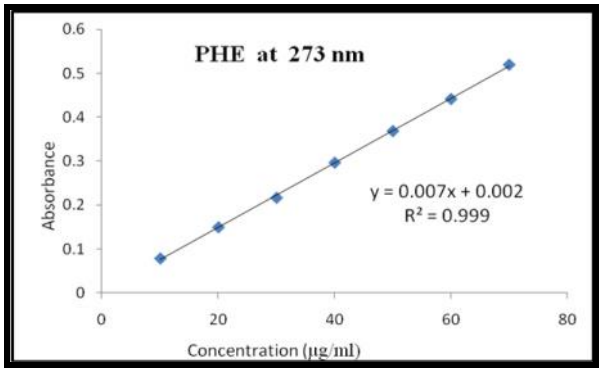


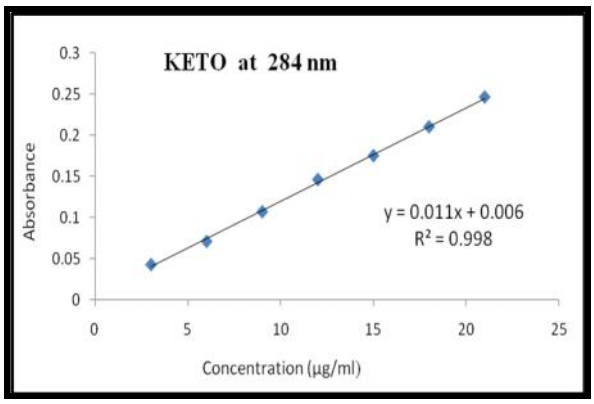
Fig. 3: Overlain Spectra of PHE and KETO



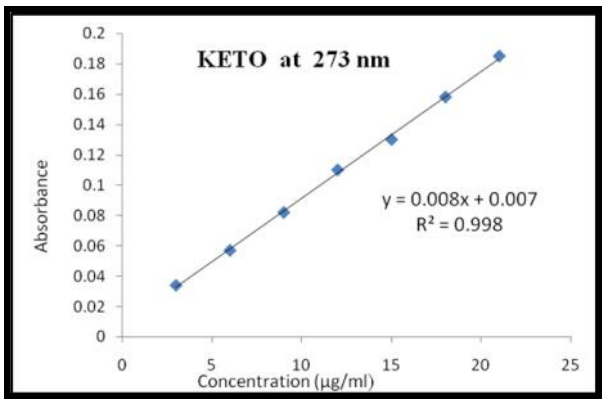
Graph 1: Calibration curve of PHE at 284 nm



Graph 2: Calibration curve of PHE at 273 nm



Graph 3: Calibration curve of KETO at 284 nm



Graph 4: Calibration curve of KETO at 273 nm

Table No. 1: Regression Analysis Data of PHE and KETO

Parameters	PHE		KETO	
Wavelength (nm)	284	273	284	**273**
Beer's law limit (µg/ml)	10-70	10-70	3-21	**3-21**
Regression equation Y = mx + c	y = 0.003x - 0.002	y = 0.007x + 0.002	Y= 0.011x + 0.006	**Y= 0.008x + 0.007**
Slope	0.003	0.007	0.011	**0.008**
Intercept	0.002	0.002	0.006	**0.007**
Correlation coefficient (r2)	**0.998**	**0.999**	**0.998**	**0.998**

Table No. 2: Repeatability of PHE and KETO

Sr. no.	PHE		KETO	
	Conc. (µg/ml)	Absorbance	Conc. (µg/ml)	Absorbance
1	9	0.082	30	0.106
2	9	0.083	30	0.106
3	9	0.083	30	0.105
4	9	0.082	30	0.107
5	9	0.084	30	0.106
6	9	0.082	30	0.105
Mean		**0.082**		**0.105**
SD		**0.000516**		**0.000753**
%RSD		**0.6272**		**0.7112**

Table No. 3: Intraday precision of PHE and KETO

Drug	Target conc.(µg/ml) (n=3)	Mean abs.	SD	%RSD
KETO (284nm)	9	0.108	0.00055	0.5177
	12	0.145	0.0010	0.6896
	15	0.174	0.00147	0.8432
KETO (273nm)	9	0.082	0.00051	0.6225
	12	0.110	0.00080	0.7339
	15	0.131	0.00115	0.8792

Drug	Target conc.(µg/ml) (n=3)	Mean abs.	SD	%RSD
PHE (284nm)	30	0.106	0.000577	0.5429
	40	0.145	0.001102	0.7593
	50	0.181	0.000404	0.2223
PHE (273nm)	30	0.218	0.0010	0.4587
	40	0.298	0.00095	0.3189
	50	0.368	0.00057	0.1567

Table No. 4: Interday precision of PHE and KETO

Drug	Target conc.(µg/ml) (n=3)	Mean abs.	SD	%RSD
KETO (284nm)	9	0.104	0.001419	1.3547
	12	0.145	0.001528	1.0510
	15	0.174	0.001401	0.8032
KETO (273nm)	9	0.084	0.0010	1.1904
	12	0.112	0.001528	1.3598
	15	0.132	0.00205	1.5527

Drug	Target conc.(µg/ml) (n=3)	Mean abs.	SD	%RSD
PHE (284nm)	30	0.106	0.0010	0.7188
	40	0.144	0.00152	0.5166
	50	0.182	0.00145	0.4072
PHE (273nm)	30	0.218	0.001572	0.9433
	40	0.295	0.001528	1.0558
	50	0.367	0.001498	0.7945

Table No. 5: LOD & LOQ of PHE and KETO

Parameters	KETO		PHE	
	273	284	273	284
Standard Deviation	0.000516	0.000837	0.001506	0.000753
Slope	0.008	0.011	0.007	0.003
LOD (µg/ml)	0.2130	0.2509	0.7097	0.8280
LOQ(µg/ml)	0.6454	0.7606	2.1507	2.5092

Table No. 6: Accuracy of PHE and KETO

Drug	% Spiking	Conc. of test taken (µg/ml) (n=3)	Conc. of std added (µg/ml)	Total conc found (µg/ml)	Calculated spiking (µg/ml)	% Recovery
KETO	80	9	7.2	16.20	16.12	99.53
	100	9	9	18	17.85	99.16
	120	9	10.8	19.80	19.75	99.74
PHE	80	30	24	54	54.14	100.20
	100	30	30	60	59.85	99.76
	120	30	36	66	66.18	100.27

Table No. 7: Assay of KETO and PHE

Tablet	Label claim	Amount found	% Drug
PHE	3	3.01	100.5
KETO	10	10.11	101.1

CONCLUSION

Based on the results, it can be concluded that the method has linear response in the range of 10-70 and 3-21 µg/ml for Phenylephrine Hydrochloride and Ketorolac Tromethamine. Less than 2 % RSD indicate that UVspectroscopic methods are accurate and precise. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with prepared ratio of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Phenylephrine Hydrochloride and Ketorolac Tromethamine .The method can be used for the routine analysis of Phenylephrine Hydrochloride and Ketorolac Tromethamine in dosage form.

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REFERENCES:

1. Ketorolac Tromethamine", http://www.drugbank.ca/drugs/DB00465
2. phenylephrine HCL", http://www.drugbank.ca/drugs/DB00388
3. Indian Pharmacopoeia, I Govt. of India, Ministry of Health & Family welfare, The Controller of Publication, Ghaziabad, **2014**; Vol II: pp 2037, 2478.
4. Beckett AH., and Stenlake JB. Practical Pharmaceutical Chemistry; 4th Edn; Part-II C.B.S Publishers and Distributors, New Delhi**, 2007**; pp 285-297.

5. Skoog DA., Holler FJ and Nieman TA. Introduction to UV Spectroscopy in Principle of instrumental analysis; 5th Edn; Thomson Brooks/Cole publication, pp 301.
6. Vandana P, Subhash J, Sachidanand S, Suwarna H and Shital D. *Asian j. of Res. in Chemistry*, **2014**; 7: pp. 19-24.
7. Bhavsar DN, Thakor NM, Patel C, Shah VH and Upadhyay UM. *Int. J. of Res. in pharm. & sci.*, **2013**; 3(2): pp. 146-153.
8. Bhatt YJ, Sharma SK and Multani P. *Int. J. of Pharm. Sci. Review and Res.*, **2013**; 20(2): pp. 118-120.
9. Suratiya S, Bhati S, Patel A, Patel G and Pancholi S. *Ame. J. of Ph. Tech Res.*, **2014**; 4(2): pp. 224-233.
10. Anandakumar K and Veerasundari P. "*Hindawi Publishing Corporation*, **2014**.
11. Wadher SJ, Kalyankar TM and Panchal PP. *Int. J. of Chem Tech Res.*, **2013**; 5: pp. 2410-2419.

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