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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF KETOROLAC TROMETHAMINE AND PHENYLEPHRINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A RP-HPLC method were developed and validated for simultaneous estimation of Phenylephrine Hydrochloride (PE) and Ketorolac Tromethamine (KETO). The separation was achieved on an Enable C18 (250mm × 4.6mm i.d., 5µm particle size) with an isocratic system of Methanol: Phosphate buffer 0.1% TEA (pH 3.3 by OPA) in the ratio of 55:45v/v. The retention time for PHE and KETO was obtained as 3.690 min and 5.890 min respectively with a flow rate of 1.0ml/min at detection wavelength 284 nm. The linearity of the proposed method was investigated in the range of 10-50 µg/ml and 3-15 µg/ml for PHE and KETO respectively. The developed method was validated as per ICH guideline and found to be satisfactory.

KEYWORDS: Ketorolac Tromethamine (KETO), Phenylephrine Hydrochloride (PHE), RP-HPLC, Validation.

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.[1,2,3]

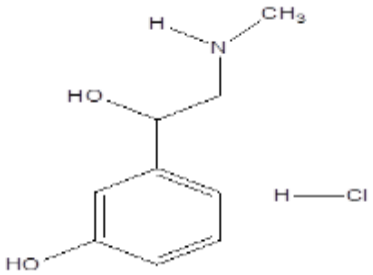

Figure 1: Structure of phenylephrine hydrochloride.

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.[1,2, 3]

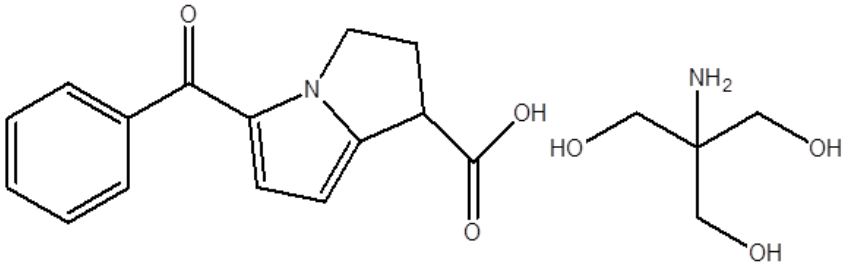

Figure 2: Structure of ketorolac tromethamine.

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 chromatographic method is reported for simultaneous estimation of Phenylephrine and Ketorolac in Pharmaceutical dosage form. Therefore, in the present work; a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC method.

MATERIALS AND 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). Methanol, Orthophosphoric acid and Triethylamine HPLC grade were procured from MERK.

Selection of detection wavelength

PHE and KETO prepared in Distilled water. These drug solutions were than scanned in the UV region of 200-400 nm and the overlain spectrums were recorded and final wavelength 284 nm is selected for detection of the drugs.

Selection of mobile phase

Resolution is the most important criteria for the method, it is imperative to achieve good resolution among the compounds. As per the value of pKa and solubility of compound various composition of mobile phase were tried. Method development for separation of KETO and PHE in combination was started with HPLC grade methanol, acetonitrile, water. The standard solutions of KETO and PHE were injected in to the HPLC system and run in solvent system. Methanol: phosphate buffer 0.1% TEA (55:45 v/v), pH was adjusted 3.3 with Ortho-phosphoric acid. Flow rate was adjusted at 1.0ml/min provides optimum resolution with good peak symmetry.

Preparation of standard stock solution of KETO & PHE

KETO Std. solution I (30 µg/ml): Accurately weighed 3mg KETO was taken in 100 ml volumetric flask and then diluted with Mobile phase up to the mark.

KETO stock solution II (3µg/ml): Prepared by transferring 1 ml from above solution to 10ml volumetric flask and then diluted with mobile phase upto the mark.

PHE std. solution I (100 µg/ml): Accurately weighed 10 mg PHE was taken in 10 ml volumetric flask and then diluted with Mobile phase up to the mark.

PHE stock solution II (10 µg/ml): Prepared by transferring 1 ml from above solution to 10ml volumetric flask and then diluted with mobile phase upto the mark.

Preparation of combined standard stock solution of Ketorolac Tromethamine and Phenylephrine hydrochloride

Accurately weighed KETO (30 mg) and PHE (100 mg) were transferred in to 100 ml volumetric flask and dissolved in 100 ml distilled water and diluted up to the mark with distilled water to give a stock solution (300 µg/ml) of KETO and (1000µg/ml) of PHE. Again 10 ml solution was withdrawn and transfer to the 100 ml volumetric flask to prepare 30µg/ml KETO and 100µg/ml PHE. This solution was used to prepare standard solution for linearity.

Validation of rp-hplc method

Linearity (Calibration curve)

Combined standard stock solution (1.0, 2.0, 3.0, 4.0 and 5.0 ml equivalent to 10, 20, 30 40 and 50µg/ml of PHE and 3, 6, 9, 12 and 15µg/ml of KETO) were transferred in a series of 10 ml volumetric flasks and diluted to the mark with mobile phase. An aliquot (20 µl) of each solution was injected under the operating chromatographic conditions as described earlier. Chromatograms were recorded. Calibration curves were constructed by plotting peak areas versus concentrations and the regression equations were calculated. Each response was average of five determinations.

Accuracy (% Recovery)

It was determined by calculating the recovery of PHE and KETO from dosage form, by standard addition method. To a fixed amount of test, 80%, 100% and 120% amount of standard was added and the amount of standard added was calculated using regression equation. Known amount of standard solutions of PHE (8, 10 and 12 µg/ml) and KETO (2.4, 3and 3.6 µg/ml) were added to a pre-quantified sample solution of PHE and KETO (10 and 3 µg/ml, respectively). Each solution was injected in triplicate and the percentage recovery was calculated by measuring the responses and fitting these values into the regression equations of the respective calibration curves.

Precision

Repeatability

6 replicates of standard mixture solution having PHE (10µg/ml) and KETO (3µg/ml) were prepared and chromatograms were recorded and %RSD was calculated.

Intraday precision

Standard solutions containing 10, 30 and 50 µg/ml PHE and 3, 9 and 15µg/ml KETO were analyzed 3 times on the same day as per the procedure. Chromatogram of each sample was taken. SD and %RSD were calculated.

Interday precision

Standard solutions containing 10, 30 and 50 µg/ml PHE and 3, 9 and 15µg/ml KETO were analyzed on three different days as per the procedure. Chromatogram of each sample was taken. SD and %RSD were calculated.

Robustness

To evaluate robustness of the method few parameters were deliberately varied. The parameters included variation off low rate and change in mobile phase. The change was made at 3 levels and replicate for 3times.The system suitability parameters were calculated for PHE and KETO.

Limit of Detection and Limit of quantitation

Calibration curve was repeated for 6 times and the standard deviation (SD) of the intercepts was calculated than LOD and LOQ was calculated as follow from the formula.

LOD= (3.3*SD)/Slope

LOQ= (10*SD)/Slope

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Estimation of PHE and KETO

Take 1 ml of dosage form from container in 100ml volumetric flask. Add mobile phase and shake well. Make it upto the mark with mobile phase (PHE 100µg/ml and KETO 30 µg/ml). The solution was sonicated for 5 minutes. The solution was filtered through 0.45 µm Whatman filter paper and first few drops of filtrate were discarded.

An aliquot (1 ml) of solution was pipette out in 10 ml volumetric flask and diluted up to mark with mobile phase to obtain solution containing 10µg/ml of PHE and 3µg/ml of KETO respectively. Chromatogram of this solution was taken and amount of PHE and KETO was calculated using regression equation.

RESULTS AND DISCUSSION

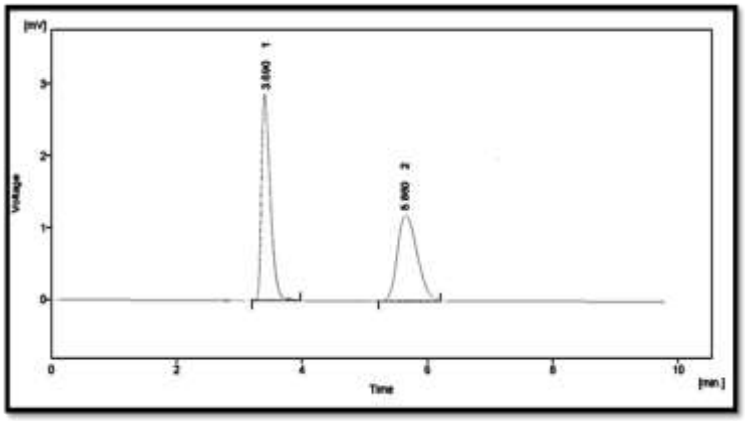

Figure 3: Chromatogram of PHE (10ppm) and KETO (3ppm) in combination.

Table 1: Finally optimized chromatographic conditions.

Parameters	Conditions
Stationary phase	Enable C18 (250mm X 4.6mm i.d., 5µm particle size)
Mobile phase	Methanol: Phosphate Buffer 0.1% TEA (55:45,V/V), pH-3.3 adjusted with orthophosphoric acid
Pump mode	Isocratic
Flow rate (ml/min)	1.0
Run time (min)	10.0
Volume of injection (µl)	20
Detection wavelength (nm)	284nm
Retention time (min)	PHE:3.690 min KETO: 5.890 min

Table 2: System suitability parameters.

Parameters	Proposed method		Standard value
	PHE	KETO	
Retention time (R_t)	3.690	5.890	-
Resolution (R_s)	-	11.034	R_s> 2
Theoretical plates (N)	3297	5455	In general should be > 2000
Tailing factor (T)	1.44	1.01	T of </= 2

Table 3: Linearity data of PHE and KETO.

Sr. No.	Concentration (µg/ml)		Peak area	
	PHE	KETO	PHE	KETO
1	10	3	58081	25712
2	20	6	120345	5356
3	30	9	167243	71345
4	40	12	226324	94668
5	50	15	282567	113558

Calibration curves

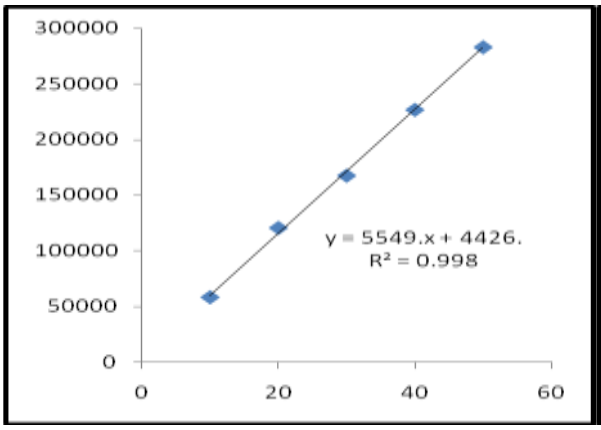

Figure 4: Calibration curve of PHE.

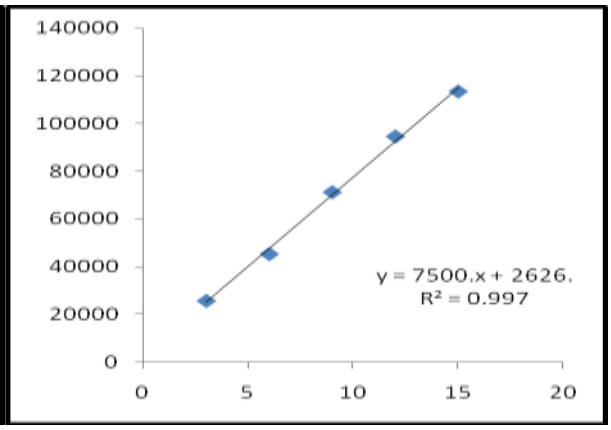

Figure 5: Calibration curve of KETO.

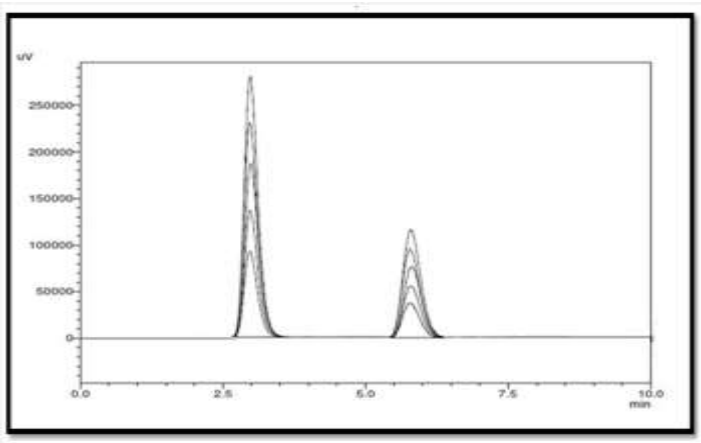

Figure 6: Overlain chromatogram of PHE and KETO.

Accuracy

Table 4: % Recovery of PHE and KETO (n=3).

Amt of sample (µg/ml)		Amt. of drug Added (µg/ml)		Amt. recovered Mean (µg/ml)		% Recovery	
PHE	**KETO**	**PHE**	**KETO**	**PHE**	**KETO**	**PHE**	**KETO**
10	3	8	2.4	17.9	5.5	99.44	101.85
10	3	10	3	19.8	5.95	99.95	99.16
10	3	12	3.6	21.85	6.59	99.31	99.84

Discussion: Result obtained reveals that % recovery of PHE and KETO was within acceptance criteria given in ICH i.e. 98-102%.

Precision

Repeatability

Table 5: Repeatability data for PHE and KETO.

Sr. No.	Drug	Conc. (µg/ml)	Mean Peak Area*	S.D.*	%RSD
1	Phenylephrine Hydrochloride	10	58147	100.3768	0.17
2	Ketorolac Tromethamine	3	25747.4	63.81066	0.24

*Average of six determinations

Intra-day precision

Table 6: Intra-day data for PHE and KETO.

Conc. (µg/ml)		Mean Peak Area*± S.D.*		%RSD	
PHE	KETO	PHE	KETO	PHE	KETO
10	3	58137±66.84	25776±69.54	0.114	0.26
30	9	1673997±169.74	71559±222.39	0.101	0.31
50	15	283020±507.85	113823±432.15	0.179	0.37

*Average of three determinations

Inter-day precision

Table 7: Inter-day precision data for estimation of PHE and KETO.

Conc.(µg/ml)		Mean Peak Area± S.D.*		%RSD	
PHE	KETO	PHE	KETO	PHE	KETO
10	3	58173±128.43	25750±87.07	0.22	0.33
30	9	167356±193.99	71615±254.31	0.11	0.35
50	15	282908±571.98	113899±564.29	0.20	0.49

*Average of three determinations

Discussion: Result obtained reveals that % RSD of PHE and KETO was within acceptance criteria given in ICH i.e. less than 2. So, the proposed method for estimation of PHE and KETO is précised in nature.

Robustness

Table 8: Robustness data for PHE and KETO.

Condition	Variation	PHE			KETO		
		Mean peak area	S.D.	% RSD	Mean Peak area	S.D.	% RSD
Mobile phase (55±5%) Methanol:	60:40 (V\V)	177376.3	164624	0.092	73639.3	1783.7	0.024
	50:50 (V/V)	215669	5820.29	0.026	110197.3	1421.2	0.013

buffer							
Flow rate (1±0.1 ml/min)	1.1 ml/min	59189	140.91	0.238	24652.6	111.55	0.452
	0.9 ml/min	177633	2032.49	0.011	73991.6	1664.7	0.022

***** In control conditions mobile phase: Methanol: Phosphate buffer 55:45 v/v at pH 3.3 with 1.0 ml/min flow rate.

Discussion: Evaluation of robustness study was carried out by calculating %RSD of system suitability parameters such as area, tailing factor and resolution. The study suggested that all the parameters have no significant influence on the determination. Results indicate that the selected factors remained unaffected by small variation of the parameters and %RSD was less than 2, which demonstrates that the proposed method was robust.

Limit of Detection and Limit of Quantitation

Table 9: LOD and LOQ for PHE and KETO.

Drug	LOD	LOQ
PHE	0.121	0.369
KETO	0.041	0.126

Discussion: The proposed method can detect and quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

Estimation of PHE (10 µg/ml) and KETO (3 µg/ml) in Dosage Form

Table 10: Assay of PHE and KETO.

Drug	Conc. Dosage Form	Conc. taken for assay	Area of sample solution	Conc. Found (µg/ml)	%Assay ±S.D
KETO	0.3 mg/ml	3 µg/ml	25712	2.89	98.66±63.81
PHE	10 mg/ml	10µg/ml	58147	9.97	99.40±100.37

Discussion*:* % Assay of PHE and KETO was found in an acceptance limit so this method could be used for analysis of this combination.

CONCLUSION

Based on the results, it can be concluded that the method has linear response in the range of 10-50 and 3-15 µg/ml for Phenylephrine Hydrochloride and Ketorolac Tromethamine respectively. Less than 2 % RSD indicate that RP- HPLC 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 presents 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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