



RESEARCH ARTICLE

Method Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Ketorolac Tromethamine and Phenylephrine Hydrochloride in their Synthetic Mixture

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Manuscript No: IJPRS/V4/I2/00076, Received On: 27/04/2015, Accepted On: 05/05/2015

ABSTRACT

Simple, precise, accurate and reproducible first order derivative UV spectroscopic method for determination of Ketorolac tromethamine and Phenylephrine Hydrochloride in its synthetic mixture was developed. The λ_{\max} value for Ketorolac tromethamine was 322 nm and for Phenylephrine HCl was 273 nm. The Zero Crossing Points (ZCPs) for Ketorolac Tromethamine and Phenylephrine HCl were obtained at 226 nm and 295 nm respectively. Linearity ranged from 4-14 μ g/ml (Ketorolac tromethamine) and 12-42 μ g/ml (Phenylephrine HCl). The method was validated as per ICH Q2 (R1) guidelines. The proposed method can be successfully employed for routine QC analysis.

KEYWORDS

Ketorolac Tromethamine, Phenylephrine Hydrochloride, First order derivative spectroscopic method, ICH Q2 (R1) guidelines and ZCPs

INTRODUCTION

Ketorolac Tromethamine is a non-steroidal anti-inflammatory drug. The molecular formula is $C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$ with a molecular weight of 376.4 gm/mol. It is a white crystalline powder and freely soluble in water and methanol and slightly soluble in ethanol. It is a light sensitive drug.^{1,2} It is a nonselective COX inhibitor. It inhibits Prostaglandin synthesis and relieves pain by peripheral mechanism. It has potent analgesic and modest anti-inflammatory activity. Ketorolac is frequently used in postoperative, dental and acute musculoskeletal pain³. Phenylephrine HCl is a Sympathomimetic drug. The molecular formula is $C_9H_{13}NO_2 \cdot HCl$ with a molecular weight of 203.7 gm/mol. It is a white crystalline powder and soluble in water and ethanol.^{1,4}

It is a selective α_1 agonist. It raises blood pressure by causing vasoconstriction. It also tends to reduce intraocular tension by constricting ciliary body blood vessels.

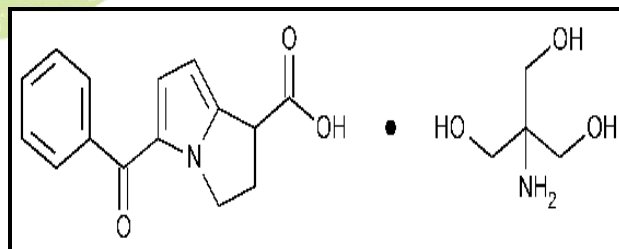


Figure 1: Structure of Ketorolac Tromethamine

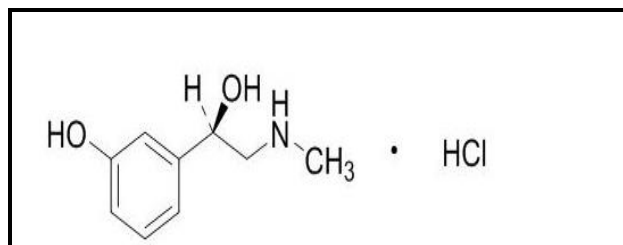


Figure 2: Structure of Phenylephrine HCl

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It is used in temporary relief of nasal congestion and pressure, and of minor eye irritations; pupil dilation in uveitis; treatment of open-angle glaucoma; diagnostic procedures and before surgery; refraction without cycloplegia³.

MATERIAL AND METHODS

Instruments

The UV used was ELICO Model SL 210, FTIR of Shimadzu 8400 series, (Shimadzu, Japan) and Analytical balance was Libror AEU-210 (Shimadzu)

Reagents

Standard Phenylephrine HCl was procured from Surya organics and chemicals, Ankleshwar, Standard Ketorolac tromethamine was procured from MSN lab Ltd, Telangana, India and distilled water was procured from E. Merck (India) Ltd., Mumbai.

Working standard solution for both the drugs was prepared in following manner.

Standard Stock Solution

100mg of drug was dissolved in a 100 ml of distilled water. The solution formed was of concentration 1000 μ g/ml.

Working Standard Solution

From the above solution 1ml was taken and diluted up to 10ml. The resulting solution was of concentration 100 μ g/ml.

Selection of Wavelength for Simultaneous Estimation of Ketorolac Tromethamine and Phenylephrine HCl⁵

The series consisting of six concentrations of standard Ketorolac tromethamine solution ranging from 4-14 μ g/ml (Ketorolac Tromethamine) and 12-42 μ g/ml (Phenylephrine HCl) were prepared from the working standard solution. Each solution was scanned and converted to first order for determining the ZCP.

Method Validation⁶

Linearity

Linearity for Ketorolac was obtained from 4-14 μ g/ml and for Phenylephrine from 12-42 μ g/ml.

Response of each solution was measured at 295 nm and 226 nm for Ketorolac tromethamine and Phenylephrine HCl respectively using first order derivative spectrophotometric method.

The graph of response versus respective concentration was plotted at 295 nm and 226 nm for Ketorolac tromethamine and Phenylephrine HCl respectively and the regression equations were calculated. Each response was average of five determinations.

Accuracy (n=3)

It was determined by calculating the recovery of Ketorolac tromethamine and Phenylephrine HCl from the synthetic mixture 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.

Each solution was scanned 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

The repeatability of the proposed method was determined by measuring the corresponding responses 6 times of Ketorolac tromethamine and Phenylephrine HCl each. The intra-day and inter-day precisions of the proposed method was determined by measuring the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of Ketorolac tromethamine and Phenylephrine HCl each.

LOD and LOQ

The LOD was estimated from the set of 5 calibration curves used to determine method linearity.

LOD was calculated as,

$$\text{LOD} = 3.3 \times (\text{SD}/\text{Slope})$$

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

The LOQ was estimated from the set of calibration curves used to determine linearity.

LOQ was calculated as,

$$\text{LOQ} = 10 \times (\text{SD}/\text{Slope})$$

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Table 1: Preparation of Synthetic Mixture (50 ml)

Ingredients	Quantity (mg)
Ketorolac Tromethamine	212
Phenylephrine HCl	620
Citric acid monohydrate	12
Sodium citrate dehydrate	274

Analysis of Synthetic Mixture

From the volume having concentration 4.24 mg/ml of Ketorolac tromethamine and 12.4 mg/ml of Phenylephrine HCl, 2 ml was taken and diluted up to the 100 ml with distilled water. From the above formed solution 1 ml was taken and diluted up to the 10 ml with distilled water. The resulting solution formed is of concentration 24.8 µg/ml (Phenylephrine HCl) and 8.48µg/ml (Ketorolac tromethamine).

RESULT AND DISCUSSION

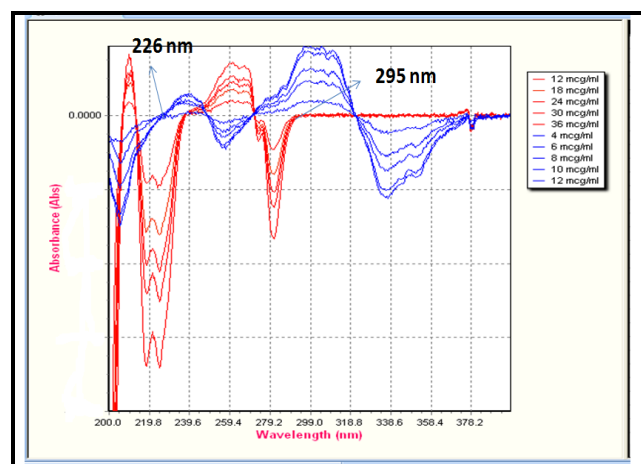


Figure 3: First order derivative overlay spectra of Ketorolac Tromethamine and Phenylephrine HCl

Table 2: Lambert-Beer's Curve Data for Phenylephrine HCl at 226 nm

Conc. (µg/ml)	Mean Response ± SD	% RSD
4	0.02162±0.000217	1.0027
6	0.02546±0.000152	0.5956
8	0.03252±0.000148	0.4561
10	0.03844±0.00027	0.7028
12	0.04412±0.000646	1.4636
14	0.04956±0.000182	0.3665

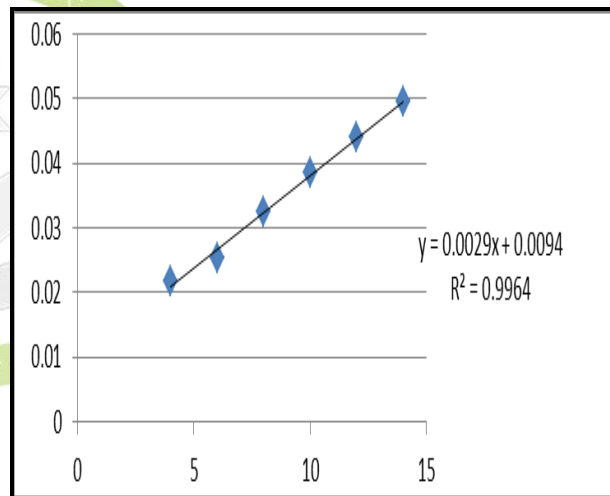


Figure 4: Calibration Curve for Ketorolac Tromethamine (4-14 µg/ml) at 295 nm

Table 3: Lambert-Beer's Curve Data for Phenylephrine HCl at 226 nm

Conc. (µg/ml)	Mean Response ± SD	% RSD
12	0.02538±0.000192	0.7578
18	0.03454±0.000288	0.8340

24	0.04188±0.000192	0.4592
30	0.05252±0.000192	0.3662
36	0.06366±0.000428	0.6719
42	0.07452±0.000319	0.4285

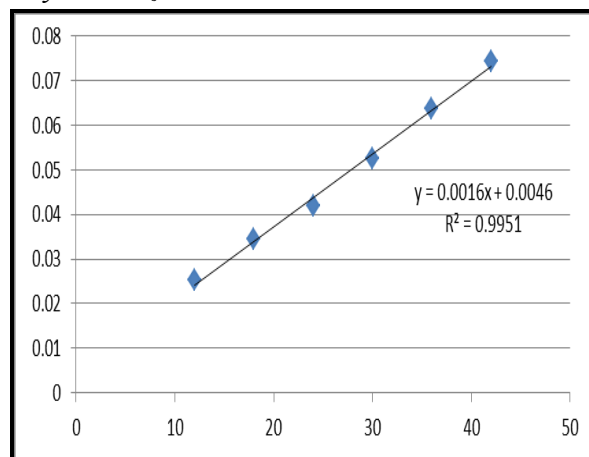


Figure 5: Calibration Curve for Phenylephrine HCl (12-42 µg/ml) at 226 nm

Table 4: Determination of Accuracy of Ketorolac Tromethamine

Amount of Ketorolac present(µg/ml)	% Amount of std Ketorolac added	Total amount of Ketorolac present(µg/ml)	Amount recoverd mean (µg/ml)	SD n=3	% Recovery
6	0	6	5.98	0.000321	99.72
	80	10.8	10.68	0.000252	98.91
	100	12	12.21	0.000208	101.80
	120	13.2	13.01	0.000252	98.61

Table 5: Determination of Accuracy of Phenylephrine HCl

Amt of Phenylephrine present (µg/ml)	% Amt of std PHEN added	Total amt of Phenylephrine present (µg/ml)	Amt recoverd mean (µg/ml)	SD n=3	% Recovery
18	0	18	17.73	0.000252	98.51
	80	32.4	32.30	0.000200	99.69
	100	36	35.40	0.000300	98.33
	120	39.6	39.70	0.000458	100.25

Table 6: Repeatability Data for Ketorolac Tromethamine and Phenylephrine HCl

Conc of Ketorolac (µg/ml)	Absorbance (n=6)	Conc of Phenylephrine (µg/ml)	Absorbance (n=6)
10	0.0384	30	0.0524
	0.0380		0.0523
	0.0385		0.0525
	0.0387		0.0527
	0.0388		0.0521
	0.0384		0.0529
Mean	0.03846	Mean	0.0524
SD	0.000280	SD	0.000286
% RSD	0.7291	%RSD	0.5445

Table 7: Data for Intraday Precision for Ketorolac and Phenylephrine

Ketorolac Tromethamine			Phenylephrine HCl		
Conc. (µg/ml)	Mean response ± SD	% RSD	Conc. (µg/ml)	Mean response ± SD	% RSD
4	0.0217±0.000153	0.7028	12	0.0255±0.000252	0.9856
8	0.0326±0.000153	0.4680	24	0.0417±0.000252	0.4784
12	0.0495±0.000152	0.3081	42	0.0745±0.000351	0.4709

Table 8: Data for Interday Precision for Ketorolac and Phenylephrine

Ketorolac Tromethamine			Phenylephrine HCl		
Conc. (µg/ml)	Mean response ± SD	% RSD	Conc. (µg/ml)	Mean response ± SD	% RSD
4	0.0218±0.00020	0.9174	12	0.0255±0.00030	1.1764
8	0.0328±0.00025	0.7657	30	0.0422±0.00036	0.8543
12	0.0494±0.00025	0.5087	42	0.0745±0.00040	0.5369

Table 9: Analysis of Synthetic Mixture (n=3)

Ketorolac Tromethamine		Phenylephrine HCl	
Amt present in synthetic mixture (mg/ml)	Amt found in assay (%)	Amt present in synthetic mixture (mg/ml)	Amt found in assay (%)
4.24	99.05	12.4	100.80
	100.82		98.38
	97.87		97.17
Mean ± SD	99.25 ± 1.483	Mean ± SD	98.79 ± 1.847
% RSD	1.495	% RSD	1.847

CONCLUSION

A new method was developed for the estimation of Ketorolac Tromethamine and Phenylephrine HCl. All the validation parameters were found in the range. So, it reveals that first order Derivative Spectroscopic Method was validated as per ICH guideline Q2 (R1).

ACKNOWLEDGEMENT

It gives me an immense pleasure to express my gratitude towards my Guide Mrs. Hetal K Patel, Mrs. Bhakti Ladva, my faculty member, MSN Lab Ltd, Surya organics and chemicals and Dr Bhavesh S. Nayak, Principal of Shree Swaminarayan College of Pharmacy for their encouragement. Their positive attitude towards work has instilled more confidence in me.

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