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Research Article

Development and Validation of Dual Wavelength Spectrophotometric methods for Simultaneous Estimation of Ketotifen and Salbutamolin in Bulk and Pharmaceutical Dosage Form

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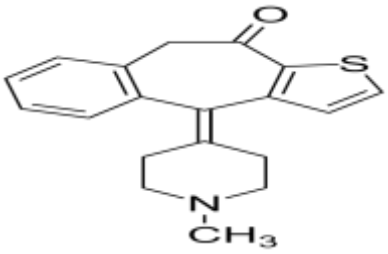
Abstract: To develop and validate a simple& accurate Spectrophotometry methods for simultaneous estimation of Ketotifen and Salbutamol in their combined pharmaceutical dosage form. Two simple, accurate, precise U.V Spectroscopy methods have been developed. First method was based on Simultaneous Equation method. Here 301 nm was selected for the estimation of Ketotifen& 276 nm selected for estimation of Salbutamol. The second method was Dual wavelength method, Here 284 nm & 267.84 nm selected for the estimation of Ketotifen where Salbutamol show same absorbance. Other 315 nm & 284.59 nm selected for estimation of Salbutamol where Ketotifen show same absorbance. Ketotifen and Salbutamol showed linearity in the range of 5-25µg/ml and 10- 50µg/ml respectively in both methods. Both methods were validated by validation parameters and it show result where lie within its acceptance criteria as per ICH Q2 (R1) guideline. Hence, it can be successfully used for the routine analysis of Ketotifen and Salbutamolin their combined pharmaceutical dosage forms.
Keywords: Ketotifen, Salbutamol, 0.1N HCLand Validation parameter.

INTRODUCTION

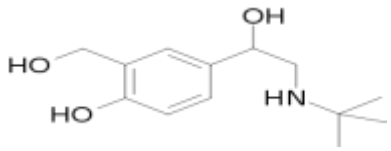
Ketotifen is used to minimize the frequency and severity of asthma attacks[1-2]. Ketotifen relieves and prevents eye itchiness and/or irritation associated with most seasonal allergies. It starts working within minutes after administering the drops. The drug has not been studied in children under 3. The mean elimination half-life is 12 hours. Its chemical name is 2-(1-methylpiperidin-4-ylidene)-6-thiatricyclo [8.4.0.0{3, 7}] tetradeca-1(10), 3(7), 4, 11, 13-pentaen-8-one. Its Molecular formula & Molecular weight are C19H19NOS & 309.42 respectively. It is Soluble in water (10 mg/ml), DMSO and 0.1N HCL. Ketotifen is a relatively selective, non-competitive histamine antagonist (H1-receptor) and mast cell stabilizer. Ketotifen inhibits the release of mediators from mast cells involved in hypersensitivity reactions. Decreased chemotaxis and activation of eosinophils have also been demonstrated. Ketotifen also inhibits cAMP phosphodiesterase.



Structure of Ketotifen

Salbutamol is a short-acting, highly selective β2-adrenergic stimulant, used in the treatment of bronchial asthma and other forms of reversible airways obstructive diseases[3]. Salbutamol is rapidly absorbed after oral administration and undergoes presystemic metabolism in the gut, oral bioavailability is 50%. Its chemical name is 4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol. Its Molecular formula & Molecular weight are $C_{13}H_{21}NO_3$ & 239.311 respectively. It's sparingly soluble in water, soluble in ethanol (96%), slightly soluble in ether. Salbutamol is a beta (2)-adrenergic agonist and thus it stimulates beta (2)-adrenergic receptors. Binding of albuterol to beta (2)-receptors in the lungs results in relaxation of bronchial smooth muscles. It is believed that salbutamol increases cAMP production by activating

adenylatecyclase, and the actions of salbutamol are mediated by cAMP [3].



Structure of Salbutamol

As per literature there already some methods were developed for single or combination of Ketotifen and Salbutamol like UV-Spectroscopy[4,5,6], Liquid Chromatography[7,8], HPLC [9-11] and UHPLC[12]. Up to now only 1st order derivative method was published for simultaneous estimation Ketotifen and Salbutamol[13]. Still now no more methods were developed on Ketotifen and Salbutamol. In case of UV spectroscopy only first order derivative method and multi component method only developed. So it was thought to developed new UV analytical methods for simultaneous estimation of Ketotifen and Salbutamol.

Here Simultaneous equation & Dual wavelengh methods were developed and validated these methods as per ICH Q2 (R1) guideline[14].

MARTIALS AND METHODS

Instrumentation, Reagents and Material

Jasco UV-1800 UV spectrophotometer, Ketotifen, Salbutamol, 0.1N HCL

SIMULTANEOUS METHOD

Determination of wavelength for measurement

2 ml of working standard solution of Ketotifen (100 µg/ml) and 4ml of working standard of Salbutamol (100 µg/ml) was diluted to 10 ml with 0.1N HCL to get 20µg/ml of Ketotifen and 40µg/ml of Salbutamol. Each solution was scanned between 200-400 nm. The spectra of each solution were obtained. Ketotifen was found 301 nm and Salbutamol was found 276 nm. Which shown in figure no. 1.

Preparation of Calibration Curve:

Calibration STD curve for Ketotifen(5-25µg/ml)

Calibration curve for Ketotifen consisted of different concentrations of standard Ketotifen solution ranging from 5-25µg/ml. The solutions were prepared by pipetting out 0.5, 1, 1.5,2 and 2.5 ml of the working standard solution of Ketotifen (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. Then spectra were measured at 301nm. The straight-line equation was determined by putting graph con VS abs. And data was recorded in table no. 1 and figure no. 2- 3.

Calibration STD curve for Salbutamol(10-50 µg/ml)

Calibration curve for Salbutamol consisted of different concentrations of standard Salbutamol solution ranging from 10-50 µg/ml. The solutions were prepared by pipetting 1,2,3,4 and 5ml of the working standard solution of Salbutamol (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. The straight-line equation was determined by putting graph con VS abs. And data was recorded in table no. 1 and figure no. 4- 5.

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 5-25 and 10-50µg/ml for Ketotifen and Salbutamol respectively six times. Absorbance of each solution was measured at 301nm and 276 nm for Ketotifen and salbutamol respectively. The correlation coefficient and regression line equations for Ketotifen and Salbutamol were determined. Linearity of 6 concentrations were measured six times and recorded in table no.2.

Precision:

Repeatability

6 replicates of 15µg/ml concentrations of Ketotifen and 30µg/ml of Salbutamol were prepared and absorbance was measured at 301 nm & 276nm of Ketotifen and Salbutamol respectively. SD and RSD were calculated and recorded in table no. 3.

Intraday Precision

Standard solutions containing 5, 10 and 15µg/ml Ketotifen and 10, 20 and 30 µg/ml Salbutamol were analyzed 3 times on the same day. Absorbance was measured at 301 nm & 276nm of Ketotifen and Salbutamol respectively. SD and RSD were calculated and recorded in table no. 4.

Interday Precision

Standard solutions containing 5, 10 and 15µg/ml Ketotifen and 10, 20 and 30 µg/ml Salbutamol were analyzed 3 times on the three different days. Absorbance was measured at 301 nm & 276nm of Ketotifen and Salbutamol respectively. SD and RSD were calculated and recorded in table no. 5.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Ketotifen and Salbutamol Sodium by standard addition method. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated and recorded in table no. 6 & 7.

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The LOD & LOQ are estimated from the set of 6 calibration curves used to determine method linearity.
LOD = 3.3 x (SD / Slope)
LOQ = 10 x (SD / Slope)

Where,

SD = the standard deviation of Y- intercept of 6 calibration curves.
Slope = the mean slope of the 6 calibration curves.
Which are shown in table no 8

Analysis of marketed formulation:

The absorbance of the sample solution was measured at 301 nm for Ketotifen and 276 nm for Salbutamol. The concentration of each drug was calculated using Simultaneous equation.This is shown in table no. 9.

DUAL WAVELENGTH METHOD

Determination of wavelength for measurement

4 ml of working standard solution of Ketotifen (100 µg/ml) and 1ml of working standard of Salbutamol (100 µg/ml) was diluted to 10 ml with 0.1N HCL to get 5 µg/ml of Ketotifen and 10 µg/ml of Salbutamol. Each solution was scanned between 200-400 nm. The spectra of each solution were obtained. Here 284 nm &267.84 nm selected for the estimation of Ketotifen where Salbutamol show same absorbance. Other 315 nm & 284.59 nm selected for estimation of Salbutamol where Ketotifen show same absorbance. Absorption differences were calculated which shown in figure no. 6.

Preparation of Calibration Curve:

Calibration STD curve for Ketotifen(5-25µg/ml)

Calibration curve for Ketotifen consisted of different concentrations of standard Ketotifen solution ranging from 5- 25µg/ml. The solutions were prepared by pipetting out0.5,1,1.5,2 & 2.5 ml of the working standard solution of Ketotifen(100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. Here 284 nm & 267.84 nm selected for the estimation of Ketotifen where Salbutamol show same absorbance. The straight-line equation was determined by putting graph con VS abs. And data was recorded in table no. 10 and figure no. 7-8.

Calibration STD curve for Salbutamol (10-50µg/ml)

Calibration curve for Salbutamol consisted of different concentrations of standard Salbutamol solution ranging from 10-50µg/ml. The solutions were prepared by pipetting 1,2,3,4 & 5ml of the working standard solution of Salbutamol (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. Here 315 nm & 284.59 nm selected for estimation of Salbutamol where Ketotifen show same absorbance The straight-line equation was determined by putting graph con VS abs. And data was recorded in table no. 10 and figure no. 9- 10.

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 5-25 and 10-50µg/ml for Ketotifen and Salbutamol respectively six times. Absorbance of each solution was measured at selected wavelength. The correlation coefficient and regression line equations for Ketotifen and Salbutamol were determined. Linearity of 6 concentrations was measured six times.

Precision:

Repeatability

6 replicates of 10 µg/ml concentrations of Ketotifenand 30 µg/ml of Salbutamol were prepared and absorbance was measured at selected wavelength. SD and RSD were calculated and recorded in table no. 10.

Intraday Precision

Standard solutions containing 15, 20& 25µg/ml Ketotifen and 30, 40 &50 µg/ml Salbutamol were analyzed 3 times on the same day. Absorbance was measured at selected wavelength. SD and RSD were calculated and recorded in table no. 10.

Interday Precision

Standard solutions containing 15, 20 & 25 µg/ml Ketotifen and 30, 40 & 50 µg/ml Salbutamol were analyzed 3 times on the three different days. Absorbance was measured at selected wavelength. SD and RSD were calculated and recorded in table no. 10.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Ketotifen and Salbutamol Sodium by standard addition method. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated.

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The LOD & LOQ are estimated from the set of 6 calibration curves used to determine method linearity.
LOD = 3.3 x (SD / Slope)
LOQ = 10 x (SD / Slope)

Where,

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

Slope = the mean slope of the 6 calibration curves.
Which are shown in table no 10

Analysis of marketed formulation:

The absorbance of the sample solution was measured at selected wavelength. The concentration of each drug was calculated. This is shown in table no. 11.

RESULT AND DISCUSSION

SIMULTANEOUS EQUATION

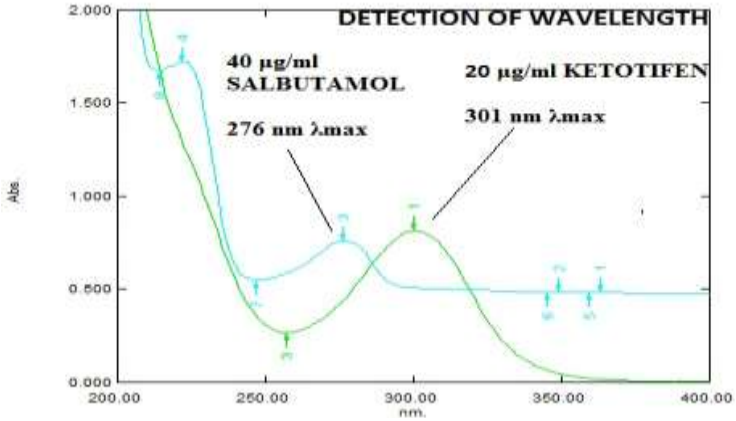


Fig-1: Determination of wavelength

Standard curve:

Table -1:Standard curve data for Ketotifen and Salbutamol

Ketotifen at 301nm		Salbutamol at 276 nm	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
5	0.23	10	0.225
10	0.408	20	0.44
15	0.600	30	0.615
20	0.813	40	0.759
25	0.987	50	0.928

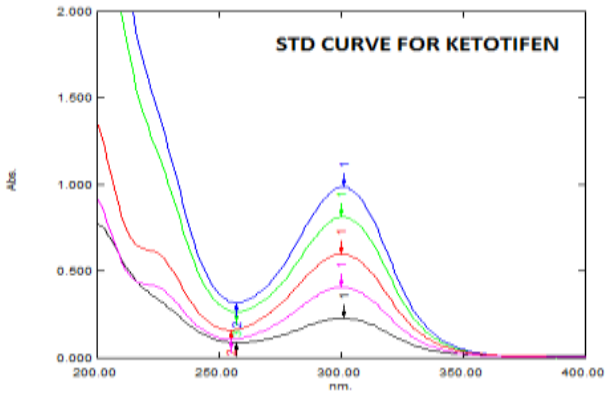


Fig-2: STD curve for Ketotifen

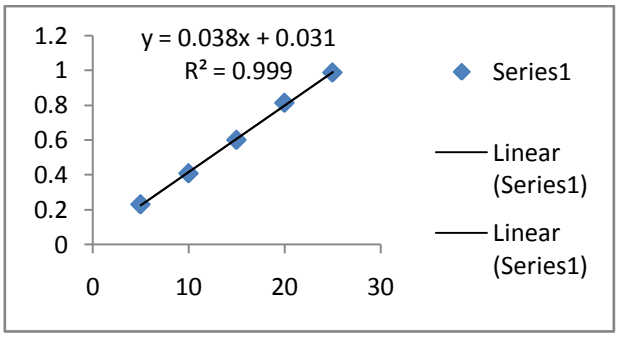


Fig-3: STD curve for Ketotifen

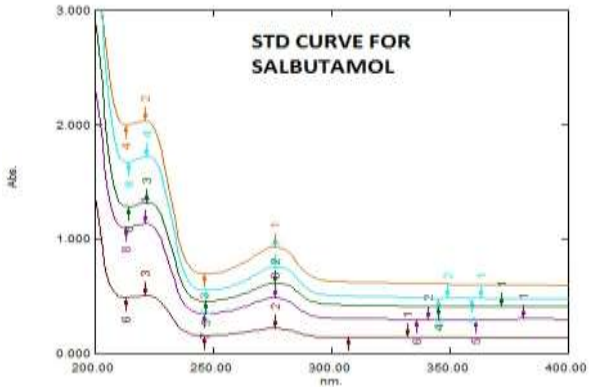


Fig-4: STD curve for Salbutamol

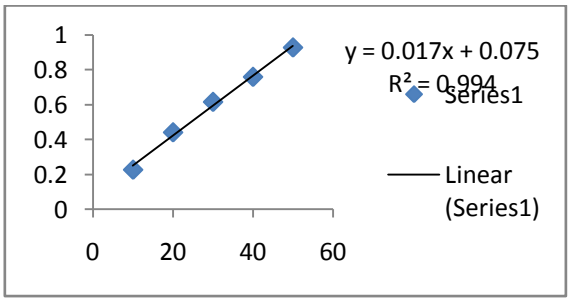


Fig- 5: STD curve for Salbutamol

Method Validation

Linearity

Table- 2: Linearity data for Ketotifen and Salbutamol

Ketotifen at 301 nm		Salbutamol at 276 nm	
Concentration (µg/ml)	**D^1Absorbance Mean* ± S.D.**	**Concentration (µg/ml)**	**D^1Absorbance Mean* ± S.D.**
5	0.229±0.0027	10	0.224±0.001
10	0.408±0.00167	20	0.431±0.002
15	0.600±0.0013	30	0.615±0.002
20	0.811±0.0024	40	0.758±0.0013
25	0.987±0.0020	50	0.928±0.0015

***n=6**

Precision

Repeatability

Table-3:Repeatability data for Ketotifen at 301 nm and Salbutamol at 276 nm

Ketotifen at 301 nm		RSD	Salbutamol at 276nm		RSD
Concentration (µg/ml)	**D^1 Absorbance ± SD**		**Concentration (µg/ml)**	**D^1 Absorbance ± SD**	
15	0.599±0.0021	0.35	30	0.616±0.0031	0.50

Intraday precision

Table-4: Intraday precision data for estimation of Ketotifen and Salbutamol

Ketotifen Concentration (µg/ml)	D^1 Absorbance* ± S.D.	%RSD	Salbutamol Concentration (µg/ml)	D^1 Absorbance* ± S.D.	%RSD
5	0.229±0.00057	0.25	10	0.224±0.0015	0.68
10	0.410±0.002	0.48	20	0.429±0.0025	0.58
15	0.602±0.0025	0.41	30	0.618±0.002	0.32

*n=3

Interday precision

Table-5: Interday precision data for estimation of Ketotifen and Salbutamol

Ketotifen Concentration (µg/ml)	Absorbance* ± S.D.	%RSD	Salbutamol Concentration (µg/ml)	Absorbance* ± S.D.	%RSD
4	0.235±0.0045	1.91	2	0.220±0.0030	1.38
5	0.401±0.0055	1.38	3	0.422±0.0060	1.44
6	0.595±0.006	1.008	4	0.606±0.0070	1.17

*n=3

Accuracy

Table-6:Accuracy (%Recovery) data for Ketotifen

% Recovery ± SD	RSD
100.3067±1.3627	1.35
100.3667±1.3051	1.13
100.44±0.5026	0.49

Table-7: Accuracy (%Recovery) data for Salbutamol

% Recovery ± SD	RSD
99.88±0.5003	0.50
100.1967±1.4678	1.46
100.1333±0.3286	0.32

Limit of Detection and Limit of Quantitation

Table-8:LOD and LOQ data for Ketotifen and Salbutamol

Parameters	Ketotifen	Salbutamol
Mean Slope (n=6)	0.038383333	0.017333
SD (n=6)	0.002923525	0.002604419
LOD (µg/ml)	0.2513	0.4957
LOQ (µg/ml)	0.7616	1.5

Analysis of marketed formulation

Table-9: Analysis of marketed formulation

Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*
Ketotifen	1	0.98	98%
Salbutamol	2	2.05	102.5%

DUAL WAVELENGTH

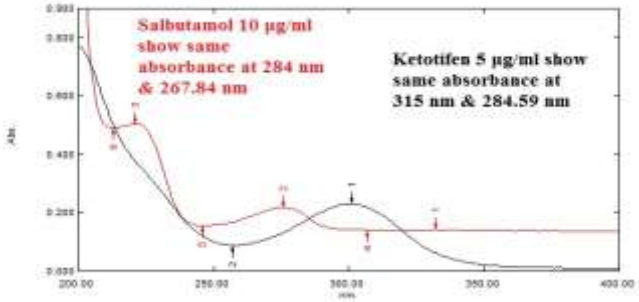


Fig-6: Determination of wavelength

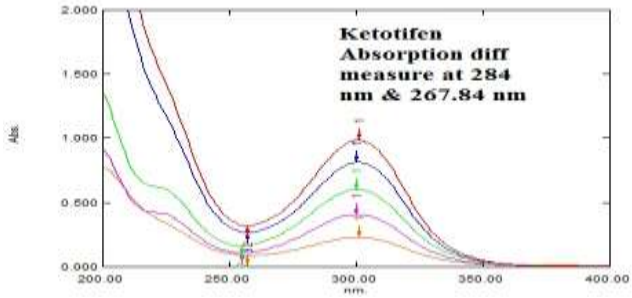


Fig-7: STD curve for Ketotifen 284 nm & 267.84

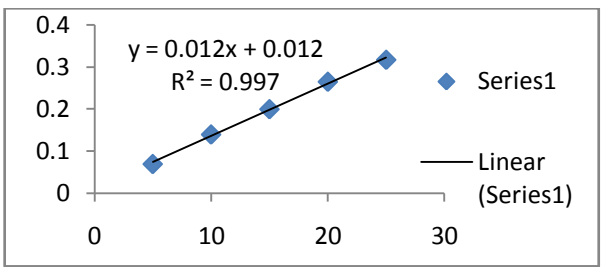


Fig-8: STD curve for Ketotifen at 284 nm & 267.84

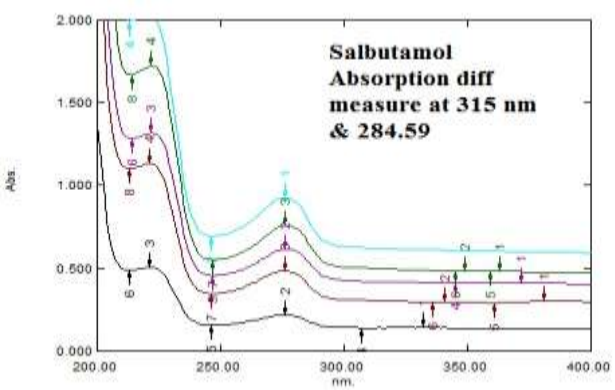


Fig-9: STD curve for Salbutamol 315 nm & 284.59

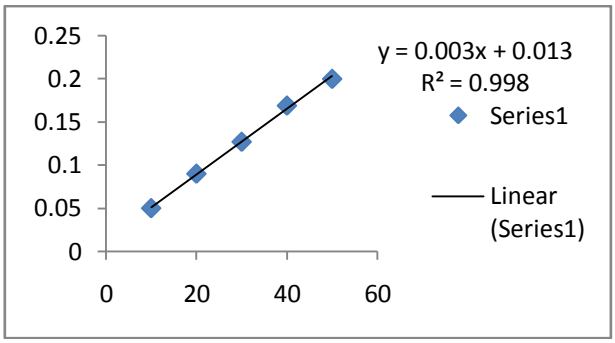


Fig-10: STD curve for Salbutamol 315 nm & 284.59

Table- 10: Summary for Ketotifen and Salbutamol for dual wavelength

PARAMETER	Ketotifen	Salbutamol
Wavelength (nm)	Absorption diff measure at 284 nm & 267.84	Absorption diff measure at 315 nm & 284.59
Beer's law limit (µg/ml) STD CURVE	5-25	10-50
Regression equation	y = 0.0124x + 0.0127	0.0038x + 0.0135
r^2	0.9978	0.9981
Slope (m)	0.0123	0.003767
Intercept (c)	0.001817	0.001335
PRECISION	INTRA DAY 0.199±0.001247 0.265±0.001247 0.318±0.001414 INTERDAY 0.196±0.002449 0.262±0.002944 0.313±0.00419	INTRA DAY 0.129±0.001155 0.170±0.001528 0.200±0.001528 INTER DAY 0.124±0.002 0.165±0.003055 0.198±0.003055
Repetability	10 µg/ml 0.200±0.001870	30 µg/ml 0.127±0.001462
LOD	0.4829	1.17
LOQ	1.46	3.5

Analysis of marketed formulation

Table-11: Analysis of marketed formulation

Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*
Ketotifen	1	1.03	103%
Salbutamol	2	1.99	99.5%

CONCLUSION

Two simple & precise UV Spectrophotometric methods have been developed and validated for the estimation of Ketotifen and Salbutamol pharmaceutical dosage form. All method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so we can conclude that methods are specific, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Ketotifen and Salbutamol pharmaceutical dosage forms.

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