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Original Article

SIMULTANEOUS ESTIMATION OF SALBUTAMOL AND KETOTIFEN IN TABLET DOSAGE FORM BY RP-HPLC USING ULTRAVIOLET DETECTION AND ITS APPLICATION FOR DISSOLUTION STUDY

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ABSTRACT

Objective: The present study aimed to develop and validate the simultaneous estimation of Salbutamol and Ketotifenin tablet dosage form.

Methods: An isocratic reversed phase high-performance liquid chromatographic (HPLC) method with ultraviolet detection at 280nm has been developed for the determination of Salbutamol and Ketotifen in dosage formulation.

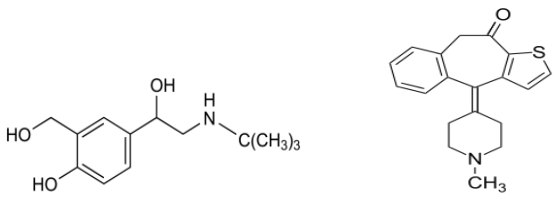
Results: Good chromatographic separation was achieved by using a Thermo Hypersil Gold ODS–C_{18} (250 mm × 4.6 mm, 5.0 µm). The system was operated at ambient temperature (25 ± 2°C) using a mobile phase consisting of Methanol: KH_2PO_4 buffer (0.025M) at pH 3.25 with ortho phosphoric acid in the ratio of 45:55 v/v at a flowrate of 1mL/minute. Linearity was observed in the concentration ranges of 10-60 µg/ml for Salbutamol and 5-30 µg/ml for Ketotifen. Percent recoveries obtained for the drugs were 99.0- 100.68 % for Salbutamol and 98.88-100.90% for Ketotifen. % RSD was found to be less than 2. The proposed method was validated for its specificity, linearity, accuracy,and precision.

Conclusion: The method was found to be suitable for the quality controlof Salbutamol and Ketotifensimultaneously in a bulk drug aswell as in a formulation.

Keywords: Salbutamol, Ketotifen, HPLC, Validation.

INTRODUCTION

Salbutamol (SAL) is chemically RS-[4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol] is a shortacting β2-adrenergic receptor agonist used for the relief of Bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease[1-3]. Salbutamol is still commonly delivered as a racemic mixture (+,-). Salbutamol, even though S-Salbutamol is known to have a detrimental effect on asthma sufferers (in fact the exact opposite effect of the R Isomer [4]. Salbutamol also has certain anti-inflammatory properties whose clinical significance is not determined [5].Salbutamol can be determined by UV[6-8], RP-HPLC[9-12], LC-MS[13] and HPTLC [14]methods have been reported for analysis of Salbutamol either alone or in combination with other drugs in pharmaceutical formulations.Ketotifen (KETO) is chemically known as 4-(1-methyl-4-piperidylidene)-4h-benzo [4, 5] cycloheptа [1, 2-b] thiophen-10(9h)-one hydrogen fumarate, is cycloheptathiophene blocker of histamine H-1 receptors used as an antiallergic and an antiasthmatic drug [15].Spectrophotometric[16-19], HPLC[20-24],LC–MS[25],HPTLC [26] and coulometric[27] methods have been reported for its determination in pharmaceutical formulations and biological fluids. The structures of the drugs are shown in Fig. 1a and 1b.



(a) (b)

Fig. 1: The chemical structures of Salbutamol (a) and Ketotifen (b)

Although the combinational use of Salbutamol and Ketotifen continuously increasing and HPLC assays offer significant economic advantages over the techniques cited above, the aim of the present investigation was to develop simple, precise and sensitive method for simultaneous determination of Salbutamol and Ketotifen in combined dosage form. The developed method is simple, precise, selective, and rapid and can be used for routine analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Salbutamol and Ketotifen were supplied, as a gift sample, from East West Pharmaceuticals Ltd, Haridwar. Mastifen-S tablet containing 2 mg Salbutamol and 1 mg Ketotifenwere obtained commercially within their shelf life. All chemicals and reagent used were of HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

Chromatographic System and Conditions

The HPLC system consisted of a pump Model Jasco PU 2080, Intelligent HPLC pump with sampler programmed at 20 µL capacity per injection. The detector UV/ VIS (Jasco UV 2075 Plus) model was used with the detection wavelength as 280 nm. The data was integrated using JascoBorwin version 1.5, LC-Net II/ADC system. The column used was Thermo Hypersil Gold ODS C_{18} column (250 × 4.6 mm, i.d., 5 µm) at ambient room temperature. The mobile phase consisted of Methanol: KH_2PO_4 buffer (0.025M) at pH 3.25 with ortho phosphoric acid in the ratio of 45:55 v/v at the flow rate of 1mL/min. The total run time was 10min. Before analysis, both, the mobile phase and the sample solutions were degassed by the use of a sonicator and filtered through 0.45µm filter paper. The identities of both the drugs were established by comparing the retention time of the sample solution with those of the standard solutions.

Preparation of Standard and Sample Solutions

The standard stock solutions of Salbutamol and Ketotifenwere prepared by accurately weighing 10 mg of each drug into a 10mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume. Further dilutions were made from this stock solution and the injection volume was kept 20µL. A calibration curve was plotted between concentrations against their respective area for both the drugs separately, and then it was found that the linearity ranges for the drugs are 10-60 µg/mL for Salbutamol and 5-30 µg/mL for Ketotifen.

Assay of Marketed Formulation

For the analysis of pharmaceutical formulation (Brand name: Mastifen-S tablet containing 2 mg Salbutamol and 1 mg Ketotifenper tablet) were assayed. Twenty tablets were crushed to a fine powder and an amount of the powder corresponding to approximately 2 mg Salbutamol and 1 mg Ketotifenwas weighed in a 25 mL volumetric flask. After addition of 15 mL methanol and sonication (30 min) the solution was diluted to volume with methanol, filtered, further dilutions were made to suitable concentration range and then injected into HPLC system for analysis.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

The drugs were soluble in solvents like water and methanol. During the development phase, the mobile phase containing Acetonitrile-water and Acetonitrile-buffer resulted in asymmetric peaks with greater tailing factor (>2) and high run time. The successful use of both Methanol and water reduced tailing and resulted in good peak symmetry and resolution. The optimized mobile phase contained Methanol: KH_2PO_4 buffer (0.025M) at pH 3.25 with ortho phosphoric acid in the ratio of 45:55 v/v at the flow rate of 1mL/min. The analytes were monitored at 280nm and the retention times were found to be 2.650 min for Salbutamol and 5.467 min for Ketotifen.

Specificity

Specificity of the method was assessed by comparing the chromatograms obtained from standard drugs with the chromatograms obtained from the tablet solutions. Because the retention times of the standard drugs and the retention times of both the drugs in sample solutions were same. The developed method was specific as no interference of excipients was found.(Fig.2)

Method Validation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges of 10-60 µg/mL for Salbutamol and 5-30 µg/mL for Ketotifen. The linear regression equations were y = 14659x – 41365, R^2 = 0.9993 for Salbutamol and y = 11619x – 16845R^2 = 0.9992 for Ketotifen. The plots obtained from linear regression and residual analysis is given in Fig. 3a and 3b for Salbutamol andFig. 4a and 4b for Ketotifen, respectively.

Limits of Detection and Quantitation

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration plot. The limits of detection (LOD) and limits of quantitation (LOQ), calculated as described above, were 5 and 8 µg/mLfor Salbutamol and 1 and 3µg/mLfor Ketotifen. This indicates the method is sufficiently sensitive.

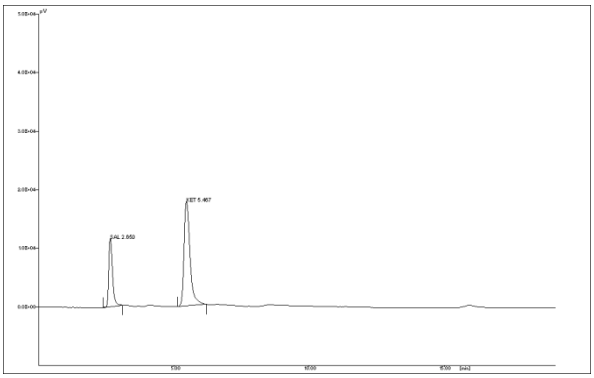


Fig. 2: Chromatogram of Salbutamol (R_t: 2.650min) and Ketotifen(R_t:5.467min)

Fig. 3a & 3b: Linear regression for Salbutamol and residual plot for Salbutamol

Fig. 4a & 4b: Linear regression for Ketotifen and residual plot for Ketotifen

Precision

The intra-day precision (% RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter- day precision (% RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.The % RSD of inter-day and intra-day precision obtained was less than 2 % for both the drugs (Table 1).

Accuracy

Analysed samples were over applied with an extra 80, 100, and 120% of the drugs from standard solutions of Salbutamol and Ketotifenand the mixtures were reanalyzed by use of the method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation. When the method was used for extraction and subsequent analysis of both the drugs from pharmaceutical dosage forms, and the extract was over applied with 80, 100, and 120% of additional drug, the recovery was listed in Table 2.

Robustness

Robustness of the method was determined by making slight changes in the experimental conditions such as the percentage of methanol use, pH of the mobile phase and flow rate of the mobile phase and the chromatographic characteristics were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstratedthat the RP-HPLC method developed, are robust. The relative standard deviation of peak areas was less than 2%. The RSD shown in Table 3indicate the robustness of the method.

Table 1: Intra-day and inter-day precision of the method

Drugs	Conc. (µg/mL)	Intra-day precision		Inter-day precision	
		Amount found (%)	% RSD	Amount found (%)	% RSD
SAL	20	99.50	1.53	99.57	0.22
	40	99.45	0.82	99.10	1.79
	60	99.39	1.05	99.67	0.58
KETO	10	100.85	1.09	99.32	1.76
	20	101.77	1.45	99.43	0.79
	30	98.94	0.18	99.71	1.36

Table 2: Results from recovery studies

Drugs	Label claim (mg/ tablet)	Amount Added(mg)	Total Amt(mg)	Amount Recovered(mg)	Recovery (%)
SAL	**2 mg**	80 % (1.6)	3.6	3.58	99.44
		100 % (2)	4.0	3.96	99.0
		120 % (2.4)	4.4	4.43	100.68
KETO	**1 mg**	80 % (0.8)	1.8	1.78	98.88
		100 % (1)	2.0	1.98	99.0
		120 % (1.2)	2.2	2.22	100.90

Table 3: Robustness of the method

Condition	SAL		KETO	
	R_t value	RSD(%)	R_t value	RSD(%)
Mobile phase composition				
Amt. of Methanol used (+)1 mL	2.5	0.23	5.5	1.96
Amt. of Methanol used (–) 1mL	2.6	0.69	5.4	1.74
Flow rate				
0.8 mL	2.7	1.56	5.5	0.23
1 mL	2.6	1.47	5.4	0.78
1.2 mL	2.5	0.96	5.3	0.84
pH				
3.0	2.7	1.47	5.5	1.26
3.2	2.6	0.85	5.4	1.58
3.4	2.5	0.77	5.3	0.11

Table 4: Result of assay of tablet formulation (n=6)

Drug	Label claim (mg/tab)	Amount found (mg)± SD	% RSD	Recovery (%)
SAL	2 mg	1.97± 0.01	0.51	98.50
KETO	1 mg	0.99± 0.01	1.01	99.00

Assay of tablet formulation

The amounts of Salbutamol and Ketotifen per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times and the result is reported in Table 4.

Dissolution Study

A calibrated Dissolution apparatus (Lab India DS-8000) was used for the dissolution study. The paddles were set at 50 rpm and the bath temperature was maintained at 37^{0}C. 450mL of distilled water was used as dissolution medium. During the dissolution study, 5mL sample was removed with replacements by maintaining the sink conditions. Samples were removed for every 5min for 45min and they were filtered through Whatman filter paper (0.42µm).

Filtrate was diluted to mobile phase Methanol: KH_2PO_4 buffer (0.025M) 45:55 (v/v) at pH 3.25 with ortho phosphoric acid and then samples were injected in the HPLC injector system. Amount of drugs in the test samples were calculated as percent dissolved from the measured peak area. Area of the test samples was taken and calculations were done by comparing them with peak area of the standard. The following formula was used:

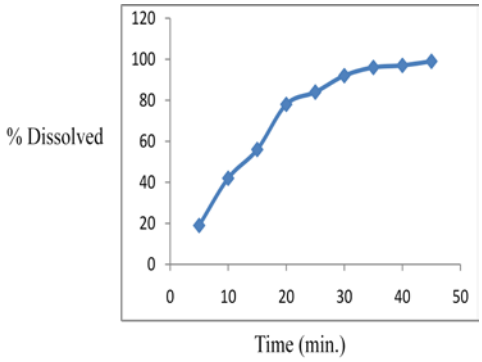
$$\% \text{ Dissolved} = \frac{\text{Area of test sample}}{\text{Area of Standard}} \text{ X } 100$$

Table 5a: Dissolution study data for SAL

Time in min	% Dissolved
5	19
10	42
15	56
20	78
25	84
30	92
35	96
40	97
45	99

Table 5b: Dissolution study data for KETO

Time in min	% Dissolved
5	24
10	45
15	58
20	70
25	82
30	88
35	92
40	95
45	98



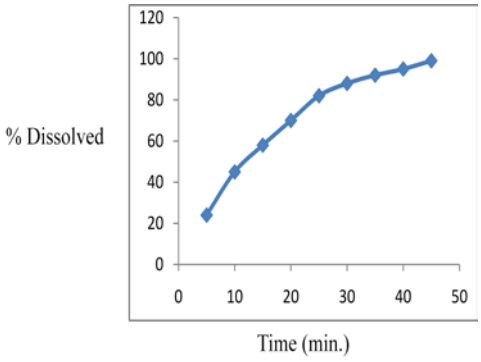


Fig. 5a: Dissolution Profile of SAL Fig.5b: Dissolution Profile of KETO

CONCLUSION

In the present study, the reversed phase HPLC (RP-HPLC) method has been developed for the estimation of Salbutamol and Ketotifenin tablet dosage form. The proposed method is simple, precise, and accurate and does not suffer from any interference due to common excipients. It is evident from the study that the method is simple, precise, specific, and accurate. The developed method can be used in the pharmaceutical industry for the routine analysis of Salbutamol and Ketotifen simultaneously, in the tablet dosage form.

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