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Research Article

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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF BAMBUTEROL IN FORMULATIONS

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ABSTRACT

A simple, accurate RP-HPLC method was developed and validated for rapid analysis of Bambuterol in formulation. Isocratic elution at a flow rate of 1.2ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at room temperature. The mobile phase consisted of Methanol: ACN: TEA 30:67:3 (V/V). The UV detection wavelength was 253 nm and 20µl sample was injected. The retention time for Bambuterol was 6.2 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for regular analysis of Bambuterol in tablet dosage form and bulk drug.

Key words: Bambuterol, RP-HPLC, UV detection, recovery, precise, 253 nm

INTRODUCTION

Bambuterol is a long acting beta-adrenoceptor agonist (LABA) used in the treatment of asthma; it also is a prodrug of terbutaline. Bambuterol is used in the long-term management of persistent asthma.[1] It should not be used as a rescue medication for short-term relief of asthma symptoms. Bambuterol is contraindicated in pregnancy and in people with seriously impaired liver function. It can be used by people with renal impairment, but dose adjustments are necessary.[1] The side effects include fatigue, nausea, palpitations, headache, dizziness and tremor.[1] Bambuterol acts as a cholinesterase inhibitor, and can prolong the duration of action of suxamethonium (succinylcholine) and other drugs whose breakdown in the body depends on cholinesterase function.[1] It can also enhance the effects of non-depolarizing neuromuscular blockers, such as vecuronium bromide.

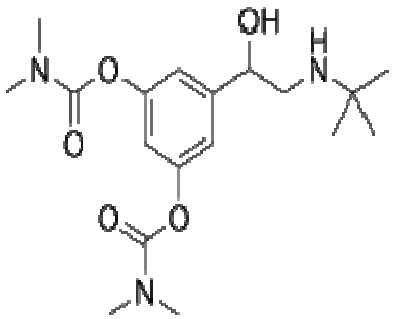


Figure.1: Chemical structure

EXPERIMENTAL

Materials

Pure form of Bambuterol was obtained from ZEN pharma, Gujarath. HPLC grade water, methanol, Acetonitrile ,Triethylamine was purchased from E. Merck.

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), A manual Rheodyne injector with a 20 µl loop was used for the injection of sample., PEAK LC software were used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Bambuterol were scanned in the range of 200 -400 nm methanol as a blank. The m maximum absorbance was found that 253 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil 100-5 C18. 250×4.6mm, , manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software were used.

The mobile phase consisted of a Acetonitrile, Methanol, Triethylamine 67:30:3 (v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1.2 ml/min. Detection was performed at 253 nm with 10 min runtime.

Standard and sample solutions

To prepare standard solution for analysis, 10 mg amount of Bambuterol API was accurately weighed, dissolved in10ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. From standard solution by the serial dilution we prepared required concentrations of 100ppm.2.5ml of above sample was taken and diluted to 10ml using mobile phase. A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Bambuterol was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 20 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 25 µg/ml.

Method validation

Method validation was performed under ICH guidelines.

RESULTS AND DISCUSSION

System Suitability

The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Table.1 System suitability parameters

Mobile phase	Acetonitrile:methanol:Triethylamine 67:30:3 (v/v)
Pump mode	Isocratic
pH	5.5
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	253nm
Injection Volume	20 µl
Flow rate	1.2 ml/min
Run time	10 minutes
Retention Time	6.2 minutes

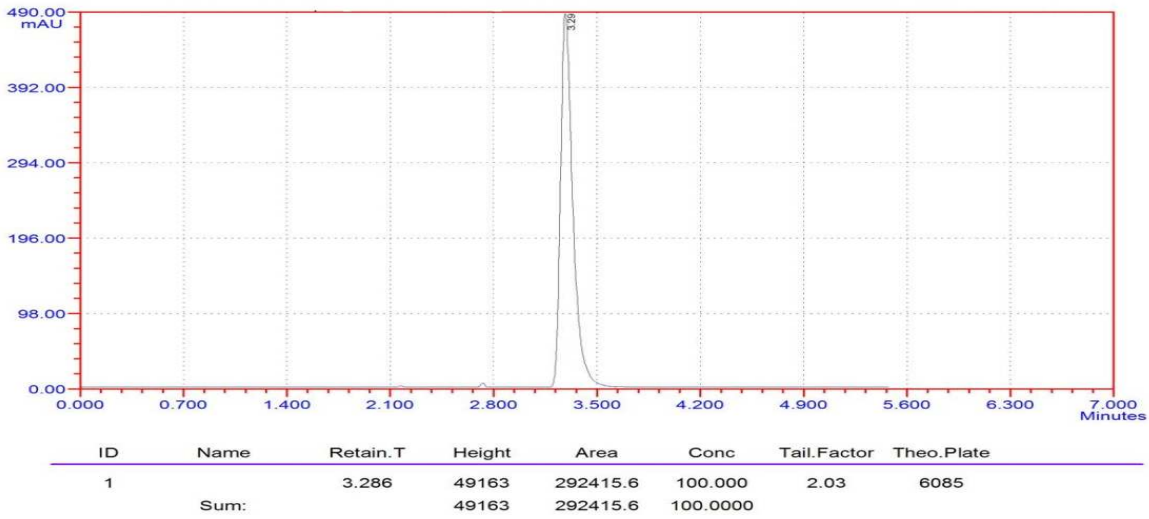


Figure.2

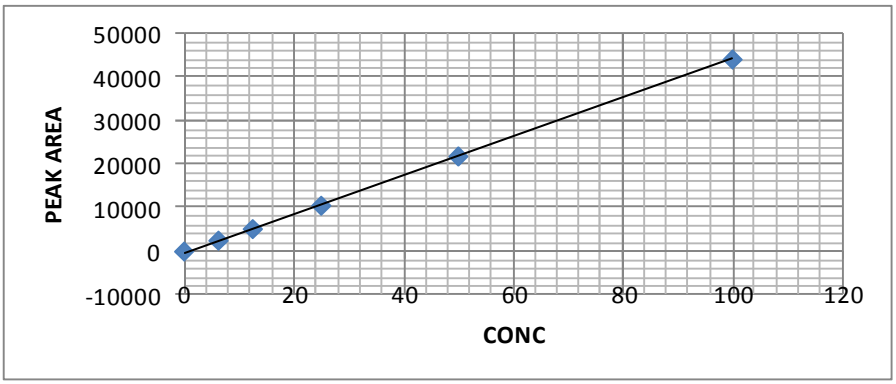
Range of linearity

The linearity test was performed in between range 6.25, 12.5, 25, 50, 100µg/ml. for Bambuterol. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 42598 + 6031x (r= 0.997). Linearity values can shown in Table: 2

Table.2: Linearity Results

Level	Concentration of Bambuterol In ppm	peak area
Level - 1	6.25	2508
Level - 2	12.5	5186
Level - 3	25	10573
Level - 4	50	21897
Level - 5	100	44198
Range:6.25ppm to 100ppm	**Slope** **Intercept** **Correlation coefficient**	443.85 272.4 0.9999

Graph.1: Linearity Graph



Precision

Six replicate standard solutions of Bambuterol (25ppm) were prepared and analyzed using the proposed method to perform Precision test.. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be 0.89which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3

Precision Results for Bambuterol:

Table.3: Precision Results

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTRADAY RSD (Acceptance criteria ≤ 2.0%)
Bambuterol	25	1	10546	**0.60**
		2	10641	
		3	10473	
		4	10552	
		5	10639	
		6	10575	
Sample	**Conc. (in ppm)**	**Injection No.**	**Peak Areas**	**INTERDAY RSD (Acceptance criteria ≤ 2.0%)**
Bambuterol	25	1	10493	**0.32**
		2	10526	
		3	10439	
		4	10525	
		5	10464	
		6	10483	

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.02 ppm dilution Peak was not clearly observed, based on which 0.02 ppm is considered as Limit of Detection and Limit of Quantification is 0.06 ppm

Table.4: LOD & LOQ results.

Parameter	Measured Value
Limit of Quantification	0.06 ppm
Limit of Detection	0.02 ppm

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Table.5

S.NO	PARAMETER	CONDITION	AREA	% of Change
1	Standard	Standard conditions	10573	
2	Mobile phase	Acetonitrile:methanol:Triethylamine 77:20:3(v/v)	10695	101.15
3	Mobile phase pH	5.3	10543	99.71
4	Wavelength	251 nm	10637	100.60

Recovery

Recover test was performed at 3 different concentrations i.e. 12.5ppm, 25ppm, 50ppm. Results are given in table.6

Table.6: Recovery Results

RECOVERY	CONC OF SAMPLE	RECOVERY	% OF RECOVERY
50%	12.5PPM	12.48	104
100%	25PPM	24.89	99.56
150%	50PPM	50.06	100.12

FORMULATION ANALYSIS

Table.7: Analysis Results

S.NO	Formulation	Dosage	Sample conc.	Sample	% of Drug
1	MOTELUKAST	10mg	40 ppm	39.92ppm	99.8

CONCLUSION

The proposed method for the assay of Bambuterol in formulations is very simple, rapid. It can be use for regular analysis in industries.

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