Journal of Pharmacreations

PharmaCreations

ISSN: 2348-6295

Pharmacreations | Vol.5 | Issue 3 | July-Sep- 2018

Journal Home page: www.pharmacreations.com

Research article *Open Access*

To develop new RP HPLC method for the simultaneous estimation of salbutamol and beclomethazone dipropionate in pharmaceutical dosage form

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ABSTRACT

A simple and selective LC method is described for the determination of Salbutamol and Beclomethazone. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of Methanol, 40 volumes of Acetonitrile and 20 volumes of Water with detection of 230 nm. Linearity was observed in the range 20-60 µg /ml for Salbutamol (r^2 =0.998) and 10-30 µg /ml for Beclomethazone (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

INTRODUCTION

Beclomethasone dipropionate

Beclomethasone dipropionate is a prodrug of the free form, Beclomethasone (beclomethasone-17-monopropionate). An anti-inflammatory, synthetic corticosteroid, it is used topically as an anti-inflammatory agent and in aerosol form for the treatment of asthma and allergic rhinitis (seasonal and perennial). Beclometasone dipropionate is also being investigated for oral treatment in mild-to-moderate Crohn's disease of ileal or ileal-right colonic localisation and for "topical" use mild-to-moderate graft versus host disease. It is marketed under several brand names such as Qnasl (US) and Rivanase AQ [1-4]

IUPAC name

2-[(1R,2S,10S,11S,13S,14R,15S,17S)-1-chloro-17-hydroxy-2,13,15-trimethyl-5-oxo-14-(propanoyloxy)tetracyclo[8.7.0.0^{2},^{7}.0^{11},^{15}]heptadeca-3,6-dien-14-yl]-2-oxoethyl propanoate

Categories

- Adrenal Cortex Hormones
- Agents to Treat Airway Disease
- Anti-Asthmatic Agents
- Anti-Inflammatory Agents
- Corticosteroids, Dermatological Preparations

- Corticosteroids, Plain

Molecular weight

521.042

Chemical formula

$C_{28}H_{37}ClO_7$

SALBUTAMOL

Salbutamol is a short-acting, selective beta2-adrenergic receptor agonist used in the treatment of asthma and COPD. It is 29 times more selective for beta2 receptors than beta1 receptors giving it higher specificity for pulmonary beta receptors versus beta1-adrenergic receptors located in the heart. Salbutamol is formulated as a racemic mixture of the R- and S-isomers. The R-isomer has 150 times greater affinity for the beta2-receptor than the S-isomer and the S-isomer has been associated with toxicity. This lead to the development of levalbuterol, the single R-isomer of salbutamol. However, the high cost of levalbuterol compared to salbutamol has deterred wide-spread use of this enantiomerically pure version of the drug. Salbutamol is generally used for acute episodes of bronchospasm caused by bronchial asthma, chronic bronchitis and other chronic bronchopulmonary disorders such as chronic obstructive pulmonary disorder (COPD). It is also used prophylactically for exercise-induced asthma.

Categories

- Adrenergic Agonists
- Adrenergic beta-2 Receptor Agonists
- Adrenergic beta-Agonists
- Adrenergics for Systemic Use
- Agents to Treat Airway Disease
- Alcohols
- Amines
- Amino Alcohols

IUPAC name

4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol

Chemical formula

$C_{13}H_{21}NO_3$

Molecular weight

239.3107

AIM & OBJECTIVE

Aim

To develop new RP HPLC method for the simultaneous estimation of Salbutamol and Beclomethazone Dipropionate in pharmaceutical dosage form.

Objective

- Solubility determination of Salbutamol and Beclomethazone Dipropionate in various solvents and buffers.
- Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation

Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Spin chrome (LC SOLUTIONS)
HPLC	Shimadzu(LC 20 AT VP)
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	Inertsil ODS 3V(250x4.6mm) 5µm

Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen Phosphate	AR Grade
Acetonitrile	HPLC Grade
Dipotassium hydrogen phosphate	AR Grade
Orthophosphoric acid	HPLC Grade

Drugs used

Salbutamol and Beclomethazone Dipropionate drugs	Gift Samples obtained from Chandra labs, Hyd.
AEROTIDE (SALBUTAMOL-400mg) (BECLOMETHASONE-200mg) Tablet dosage form	Obtained from local pharmacy

Mobile phase

A mixture of, 40 volumes of Methanol, 40 volumes of methanol and 20 volumes of Water. The mobile phase was sonicated for 10 min to remove gases.

Determination of working wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of salbutamol

10 mg of Salbutamol was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of beclomethasone dipropionate

10 mg of Beclomethasone Dipropionate was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark i.e. Salbutamol and Beclomethasone Dipropionate were soluble it was used as solvent for **λmax** determination by UV-Visible Spectroscopy.

ASSAY

Preparation of samples for assay

Preparation of mixed standard solution

Weigh accurately 10 mg of Salbutamol and 10 mg of Beclomethasone Dipropionate in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40 µg/ml of Salbutamol and 20 µg/ml of Beclomethasone Dipropionate is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Sample preparation

Weigh accurately 10 Tablets **(AEROTIDE-SALBUTAMOL-400mg, BECLOMETHASONE-200mg)** 10 mg of Salbutamol and 10 mg of Beclomethasone Dipropionate in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40 µg/ml of Salbutamol and 20 µg/ml of Beclomethasone Dipropionate is prepared by pipette out 1ml from salbutamol and 0.5ml from beclomethasone and diluting**(1+0.5)** 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Calculation

$$\%\ \mathbf{Assay} = \frac{\mathbf{AT}}{\mathbf{AS}} \times \frac{\mathbf{WS}}{\mathbf{DS}} \times \frac{\mathbf{DT}}{\mathbf{WT}} \times \frac{\mathbf{P}}{\mathbf{100}} \times \frac{\mathbf{Avg.Wt}}{\mathbf{LC}} \times \mathbf{100}$$

Where,
AT = Peak area of sample preparation,
AS = Average Peak area of standard preparation,
WS = Weight of drug in mg,
DS & DT= Dilution of standard and sample preparation,
WT = Weight of Sample in Assay preparation,
P = Percentage purity of working standard,
LC = Label Claim of drug.

Assay results

SALBUTAMOL			BECLOMETHASONE DIPROPIONATE	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2316.677	2325.488	2577.459	2568.111
Injection-2	2338.8	2333.851	2575.955	2552.59
Injection-3	2307.503	2333.143	2536.058	2559.719
Injection-4	2329.63	2332.043	2552.936	2571.49
Injection-5	2315.97	2333.75	2562.639	2574.942
Average Area	2321.716	2331.655	2561.009	2565.37
Standard deviation	3.521648		9.108415	
%RSD	0.00151		0.003551	
Assay(%purity)	100.4281	100.1703		

Observation

The amount of Salbutamol and Beclomethasone Dipropionate present in the taken dosage form was found to be 100.42% and 100.17% respectively.

METHOD VALIDATION

Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity and potency of the drug substances and drug products.

Validation parameters

a) Specificity / Selectivity
b) Accuracy
c) Precision
d) Linearity & Range
e) Limit of Detection
f) Limit of Quantitation
g) Robustness
h) Ruggedness
i) System Suitability

RESULTS AND DISCUSSION

Wavelength Optimization by UV– Spectroscopy

Method development and optimization of RP-HPLC method

Optimized chromatographic conditions

Mobile phase	**METHANOL:ACN : WATER(40:40:20)**
Ph	-
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	230
Injection volume	20 µl
Run time	6 min
Retention time	About 2.520 min for SALBUTAMOL and 5.207 min for BECLOMETHASONE DIPROPIONATE.

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both Beclomethasone Dipropionate and Salbutamol.
- The efficiency was more than 2000 Beclomethasone Dipropionate and Salbutamol.
- Resolution between two peaks >1.5.
- The details are given in the figure 8.3.8, hence this method was for optimized.

Method validation

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. Results for system suitability of SALBUTAMOL

Injection	**Retention time (min)**	**Peak area**	**Theoretical plates (TP)**	**Tailing factor (TF)**
1	2.520	2316.677	2082	1.621
2	2.517	2338.800	2076	1.621

3	2.520	2307.503	2082	1.621
4	2.533	2329.630	2104	1.679
5	2.537	2315.970	2109	1.621
Mean	2.5254	2321.716	-	-
SD	0.008961	12.39871	-	-
%RSD	0.354836	0.534032	-	-

Results for system suitability of BECLOMETHASONE DIPROPIONATE

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	5.207	2577.459	3633	1.375
2	5.187	2575.955	3605	1.375
3	5.207	2536.058	3633	1.383
4	5.207	2552.936	3633	1.383
5	5.207	2562.639	3755	1.404
Mean	5.203	2561.009	-	-
SD	0.008944	17.20732	-	-
%RSD	0.171906	0.671896	-	-

Linearity Preparations

Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				SALBUTAMOL	BECLOMETHASONE DIPROPIONATE
Preparation 1	0.5	0.25	10	20	10
Preparation 2	0.75	0.375	10	30	15
Preparation 3	1	0.5	10	40	20
Preparation 4	1.25	0.625	10	50	25
Preparation 5	1.5	0.75	10	60	30

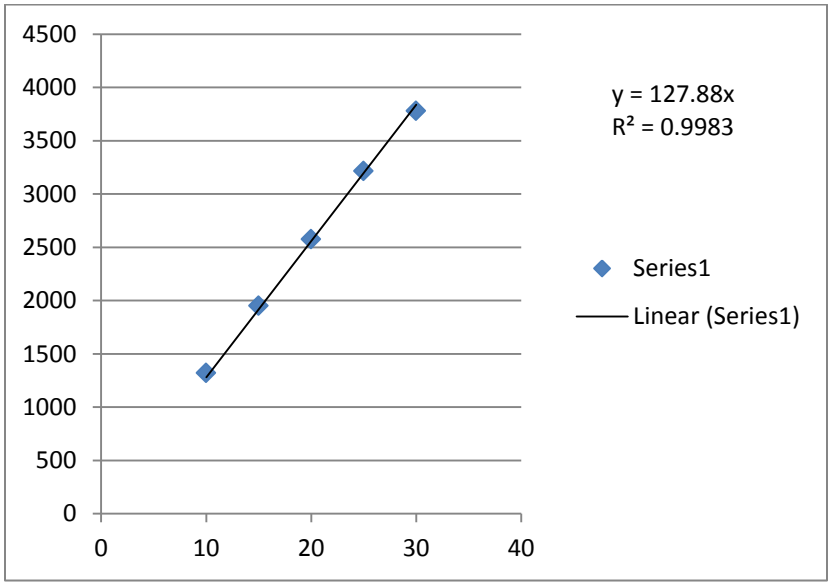
Linearity of salbutamol

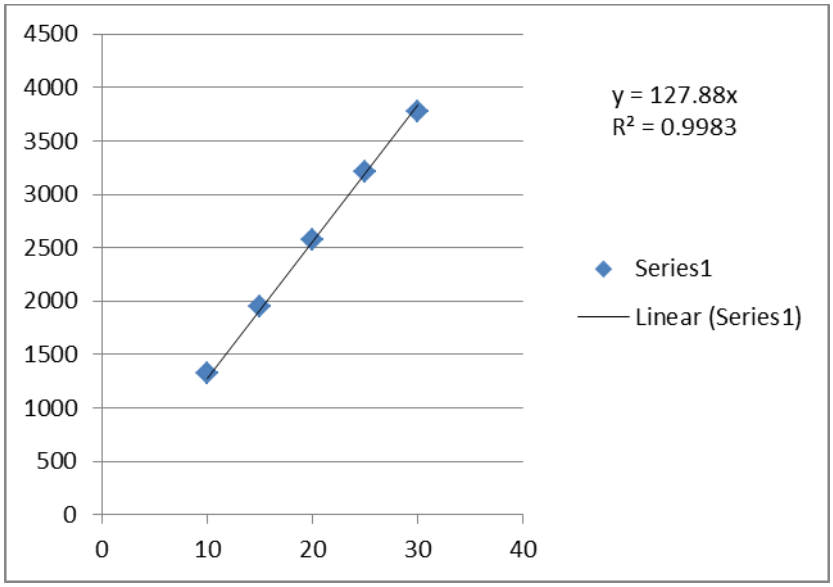
S.No.	Conc.(µg/ml)	Area
1	20	1530.419
2	30	1829.682

3	40	2325.506
4	50	2728.038
5	60	3138.275

Linearity of beclomethasone dipropionate

S.No.	Conc.(µg/ml)	Area
1	10	1681.599
2	15	2011.389
3	20	2575.330
4	25	3014.365
5	30	3479.875





Linearity graph of SALBUTAMOL

Linearity graph of BECLOMETHASONE DIPROPIONATE

Recovery results for SALBUTAMOL

Recovery level	Accuracy SALBUTAMOL					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50%	20	2319.455	2321.826	2.59	100.00	
	20	2338.467				
	20	2307.556				
100%	40	2728.038	2733.146	6.12	117.72	102.35
	40	2728.038				
	40	2743.362				
150%	60	3138.275	3142.616	7.25	89.33	
	60	3140.528				
	60	3149.047				

Recovery results for BECLOMETHASONE DIPROPIONATE

Recovery level	Accuracy BECLOMETHASONE DIPROPIONATE					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50%	10	2563.423	2569.57	7.5	100.09	
	10	2577.055				
	10	2568.237				
100%	20	3014.365	3016.010	15.25	117.70	
	20	3014.365				
	20	3019.302				102.55
150%	30	3479.875	3487.847	18.35	89,88	
	30	3488.541				
	30	3495.127				

Observation

The percentage mean recovery of SALBUTAMOL and BECLOMETHASONE DIPROPIONATE is 102.35 % and 102.55 % respectively. Results for Method precision of SALBUTAMOL and BECLOMETHASONE DIPROPIONATE

SALBUTAMOL			BECLOMETHASONE DIPROPIONATE		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	2.520	2307.922	1	5.190	2567.676
2	2.517	2310.290	2	5.203	2538.698
3	2.517	2323.689	3	5.203	2551.448
4	2.503	2310.647	4	5.177	2556.486
5	2.533	2334.444	5	5.197	2555.680
6	2.527	2330.088	6	5.190	2577.911
avg	2.5195	2319.513	avg	5.193333	2557.983
stdev	0.010232	11.40373	stdev	0.009893	13.51475
%RSD	0.406124	0.491643	**%RSD**	0.19049	0.528336

Observation

Test results for BECLOMETHASONE DIPROPIONATE and SALBUTAMOL are showing that the %RSD of Assay results are within limits. The results were shown in table Table 9.5.7.

Limit of detection

$$\text{LOD} = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

LOD of SALBUTAMOL= 0.18µg/ml

LOD of BECLOMETHASONE DIPROPIONATE =0.19µg/ml

Observation

The LOD for this method was found to be 0.18 µg/ml for SALBUTAMOL and 0.19 µg/ml for BECLOMETHASONE DIPROPIONATE

Limit of Quantification

$$\text{LOQ} = \frac{10\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

LOQ of SALBUTAMOL= 0.55µg/ml

LOQ of BECLOMETHASONE DIPROPIONATE= 0.58µg/ml

Observation

The LOQ for this method was found to be 0.55µg/ml for SALBUTAMOL and 0.58 µg/ml for BECLOMETHASONE DIPROPIONATE

Robustness

Parameter	**SALBUTAMOL**		**BECLOMETHASONE DIPROPIONATE**	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate				
0.8 ml/min	3.073	1.647	6.277	1.357
1.2 ml/min	2.533	1.679	5.197	1.426
Wavelength				
228nm	2.523	1.643	5.163	1.404
232nm	2.623	1.653	5.263	1.414

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

SALBUTAMOL	%Assay	BECLOMETHASONE DIPROPIONATE	%Assay
Analyst 01	99.09	**Analyst 01**	101.56
Anaylst 02	99.22	**Anaylst 02**	99.09

DISCUSSION

A simple and selective LC method is described for the determination of Salbutamol and Beclomethazone. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of Methanol, 40 volumes of Acetonitrile and 20 volumes of Water with detection of 230 nm. Linearity was observed in the range 20-60 µg /ml for Salbutamol (r^2 =0.998) and 10-30 µg /ml for Beclomethazone (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Salbutamol and Beclomethazone Dipropionate was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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