Available Online through
www.ijpbs.com

IJPBS |Volume 2| Issue 1 |JAN-MARCH |2012|83-98

Development and validation of first order derivative method for the simultaneous estimation of sulbutamol sulphate and Beclomethasone Dipropionate

Venkata naveen.T[1], B. Gopinath[2] and Venu.Kola[3]*

[1,2] Dept. of Pharmaceutical Analysis, National College of Pharmacy, Shimoga

[3] Dept.of pharmacology, VL College of Pharmacy, Rayachur, Karnataka.

*Corresponding Author Email:

PHARMACEUTICAL SCIENCES

Research Article

RECEIVED ON 15-12-2011

ACCEPTED ON 10-02-2012

ABSTRACT

The purpose of research is to develop and validate the sulbutamol sulphate and Beclomethasone Dipropionate by first order derivative method for the simultaneous estimation. This method is good in terms of accuracy, precision (repeatability, intermediate precision), specificity and analysis of marketed formulation. Samples are analyzed simultaneously without any separation. This method is simple. A new simple and sensitive assay has been developed for the simultaneous quantitative measurement of Beclomethasone Dipropionate and its hydrolysis products in human plasma and urine.

KEYWORDS: *simultaneous estimation, first Order derivative method.*

INTRODUCTION

Rationale for Selection of Drugs

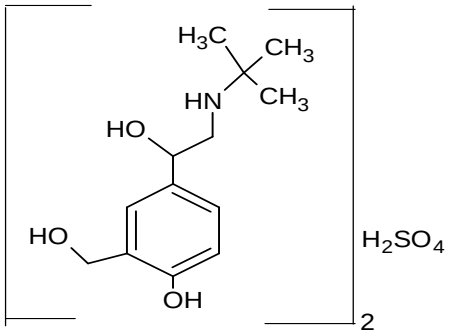
Bronchial asthma: Bronchial asthma is characterized by hyper responsiveness of trachea bronchial smooth muscle to a variety of stimuli, resulting in narrowing of air tubes, often accompanied by increased secretion, mucosal edema & mucus plugging.[7]

Salbutamol Sulphate: It is a stimulant of β_2 adrenergic receptors and it causes bronchodilation. It is used as an anti asthmatic drug. It relieves and prevents bronchospasm in patients with reversible obstructive airway disease, Prevents exercise induced bronchospasm.[7]

Beclomethasone Dipropionate: It is a steroidal drug used in asthma, it is used to control bronchial asthma in patients requiring chronic treatment, prophylaxis & treatment of allergic & vasomotor rhinitis. This drug restores responsiveness to sympathomimetic drugs like Salbutamol Sulphate once resistance to them has been developed.[7]

There may be many drugs in individual form, and in combination forms available in market, but Salbutamol Sulphate in combination with Beclomethasone Dipropionate is a good combination in market used for management of bronchial asthma.

Salbutamol Sulphate[8, 9, 10]



Mol. Formula :$(C_{13}H_{21}NO_3)_2,H_2SO_4$; Mol. Weight: 576.7; Syn: Albuterol sulphate ;C.A.S. No: 51022-70-9 ;Merck index: 215.

Salbutamol Sulphate is (RS)-1-(4-hydroxy-3-hydroxymethylphenyl)-2-(tert-butylamino) ethanol sulphate.

Physical and chemical properties:

Physical state and appearance: Solid, white or almost white crystalline powder.

Solubility: Freely soluble in water, slightly soluble in ethanol (95 %), ether and very slightly soluble in dichloromethane.

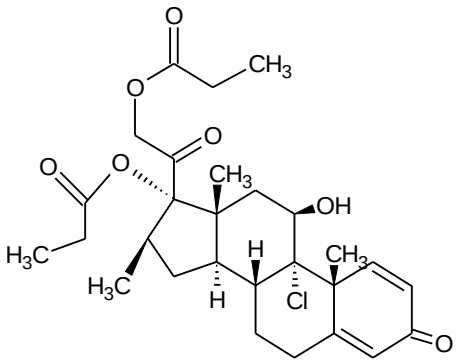
Stability: The product is stable.

Corrosivity: Non-corrosive in presence of glass.

Polymerization: Will not occur.

Storage: Store in tightly closed container protected from light.

Beclomethasone Dipropionate[8, 10, 11]



Mol.Formula: $C_{28}H_{37}ClO_7$;Mol. Weight: 521.1; Syn: Beclometasone Dipropionate; C.A.S. No: 5534-09-8 100; Merck index: 1020.

Beclomethasone Dipropionate is 9α-chloro-11β-hydroxy-16β-methyl-3, 20-dioxopregna-1, 4-iene-17, 21-diyldipropionate.

Physical and chemical properties:

Physical state and appearance**:** Solid, a white to creamy-white, crystalline powder.

Solubility: Freely soluble in acetone, chloroform, sparingly soluble in ethanol (95 %) and practically insoluble in water. Stability: The product is stable.

Corrosion: Not reported to be corrosive.

Polymerization: Will not occur.

Storage: Store in tightly closed container protected from light.

Different Spectrophotometric Methods for Multicomponent Samples.[12]

- Assay as a single-component sample
- Assay using absorbance corrected for interference
- Simultaneous equation method
- Absorbance ratio method
- Geometric correction method
- Orthogonal polynomial method
- Difference spectrophotometry
- Derivative spectrophotometry

OBJECTIVE

A. To develop first order derivative method for simultaneous estimation of Beclomethasone Dipropionate and Salbutamol Sulphate in Bulk drug and pharmaceutical formulation.
B. Validation of the method according to ICH guide lines.

METHODS REPORTED ON SALBUTAMOL SULPHATE

1. **Selective extraction of Salbutamol from human plasma with the use of phenylboronic acid.[19]**

It involves the retention of a phenylboronate–Salbutamol complex on an end-capped C_{18} solid-phase sorbent to determine the level of Salbutamol in human plasma samples. Propranolol, a β-blocker, was chosen as the internal standard for this assay. In this solid-phase clean-up method, 50 mM sodium carbonate buffer, pH 9.60, was used for conditioning the column as well as washing the endogenous interference.

2. **Determination of β-agonists in liver and retina by liquid chromatography-tandem mass spectrometry.[20]**

This procedure uses enzymatic digestion, liquid–liquid extraction, and cleanup on oasis HLB solid-phase extraction cartridges, followed by determination of the residues by LC-tandem quadrupole mass spectrometer using atmospheric pressure chemical ionization in the positive ion mode.

3. **HPLC versus SFC for the determination of Salbutamol Sulphate and its impurities in pharmaceuticals.[21]**

It uses reverse phase high performance liquid chromatography (RP-HPLC) with diode array detection (DAD). The best separation was achieved using a gradient of 0.1 M ammonium acetate pH 3.0 and acetonitrile.

4. **Determination of clenbuterol, Salbutamol, and cimaterol in bovine retina by electrospray ionization-liquid chromatography-tandem mass spectrometry.[22]**

The tissue was homogenized in alkaline buffer and spiked to give 10, 15, and 20 ng of each of the 3 analytes together with the internal standards d_6-salbutamol and d_6-clenbuterol. The mixture was incubated with protease enzyme to release any protein-bound analytes and then made alkaline before extraction with isobutanol. The extract was dissolved in water and transferred to a clenbuterol immunoaffinity column. After washing, the analytes were eluted and analyzed by

ESI/LC/MS/MS using a C_{18} column with acetic acid-methanol as mobile phase.

5. **Determination of Salbutamol in human plasma and urine by high-performance liquid chromatography with a coulometric electrode array system.**[23]

It uses using high-performance liquid chromatography (HPLC) with a coulometric electrode array system. The mobile phase component A is 30 mM sodium dihydroxy phosphate-30 mM triethylamine and is adjusted to pH 6.0 with 20 % phosphate acid. The mobile phase component B is methanol. The optimized mobile phase composition was A and B in the proportion of 90:10 (v/v). Paracetamol is selected as the external standard.

6. **Determination of Salbutamol and detection of other beta-agonists in human postmortem whole blood and urine by GC-MS-SIM.**[24]

A sensitive and quantitative method for the determination of Salbutamol and the detection of terbutaline, clenbuterol, fenoterol, and isoprenaline in postmortem human whole blood and urine. It describes solid-phase extraction, formation of trimethylsilyl derivatives, and analysis by gas chromatography-mass spectrometry-selective ion monitoring.

7. **Determination of Salbutamol in human plasma and urine by high-performance thin-layer chromatography.**[25]

Salbutamol is extracted using solid-phase techniques and converted to an indoaniline dye by reaction with dimethyl-p-phenylenediamine. The indoaniline dye is separated using HPTLC and quantified by absorption microdensitometry at 650 nm.

8. **Analysis of Salbutamol and related impurities by derivative spectrometry.**[26]

Ultraviolet derivative spectrometry has been proposed for the analysis of Salbutamol and related impurities. The assay of Salbutamol aldehyde, 5-formyl-saligenin, and Salbutamol ketone was performed in sodium hydroxide 0.1 mol/l solutions, using first and second derivative spectra.

9. **Derivatization procedures for the detection of beta (2)-agonists by GC/MS analysis.**[27]

The study was performed on the beta (2)-agonists bambuterol, clenbuterol, fenoterol, formoterol, Salbutamol, salmeterol and terbutaline. Different derivatizating agents were employed, aiming to obtain derivatives with high selectivity to be used in the gas chromatographic/mass spectrometric analysis of beta (2)-agonists in biological samples.

10. **Determination of Salbutamol in syrups by capillary electrophoresis with contactless conductivity detection.**[28]

This paper describes the separation and quantification of Salbutamol in pharmaceutical products (Salbutamol syrups) by capillary electrophoresis (CE) with contactless conductivity detection (C^4D).

METHODS REPORTED ON BECLOMETHASONE DIPROPIONATE

1. **Simultaneous determination of Beclomethasone, Beclomethasone monopropionate and Beclomethasone Dipropionate in biological fluids using a particle beam interface for combining liquid chromatography with negative-ion chemical ionization mass spectrometry.**[29]

A new simple and sensitive assay has been developed for the simultaneous quantitative measurement of Beclomethasone Dipropionate and its hydrolysis products in human plasma and urine. Beclomethasone 17, 21-dipropionate, Beclomethasone 17-monopropionate, Beclomethasone and the internal standard, dexamethasone 21-acetate, were measured by combined liquid chromatography and negative-ion chemical ionization mass spectrometry with methane as the reagent gas.

2. **The measurement of Beclomethasone Dipropionate entrapment in liposomes: a comparison of a microscope and an HPLC method.**[30]

The purpose of this study was to examine the methodologies that may be used to estimate the maximum incorporation (< 5 mole % drug) of Beclomethasone Dipropionate (BDP) in dipalmitoylphosphatidylcholine (DPPC) multilamellar liposomes.

3. **Screening for anabolic steroids in doping analysis by liquid chromatography/electrospray ion trap mass spectrometry.**[31]

A fast and selective LC/MS/MS method for the screening of four anabolic steroids in human urine

has been developed and validated. Liquid-liquid extraction with diethyl ether was applied after enzymatic hydrolysis. Analyses were performed on an ion trap mass spectrometer equipped with electrospray ionisation.

4. **HPLC determination of Beclomethasone Dipropionate and its degradation products in bulk drug and pharmaceutical formulations.**[32]

An HPLC method for the simultaneous determination of Beclomethasone Dipropionate and its principal degradation products has been developed. The only sample treatment necessary for the analysis is its dilution with methanol.

5. **Detection of corticosteroids in injection sites and cocktails by MS^n.**[33]

For the analysis of injection sites and of suspect cocktails (found at the farm), a multiple mass spectrometric (MS^n) method was developed. The method is based on rapid extraction of the matrix with methanol and direct infusion of the extract into the interface of the mass spectrometer.

6. **LC/MS-MS method for the determination of Beclomethasone Dipropionate and Beclomethasone-17-monoproprionate in human plasma.**[34]

1ml of a mixture of EDTA human plasma and internal standard (budesonide) was extracted with tert-butylmethylether. After evaporation and reconstitution in injection solvent, the organic phase was injected onto a synergi fusion-RP column with 4 µm particle size, 100 x 2.00 mm I.D. (Phenomenex). Elution was by a gradient of 2 mM ammonium acetate in a mixture of acetonitrile/high purity water containing 0.2 % formic acid at a flow rate of 0.3 ml/min.

7. **On-line high-performance liquid chromatography method for analyte quantitation from pressurized metered dose inhalers.**[35]

For the MDI systems in this study, an acetonitrile-water (90:10, v/v) mobile phase at a flow rate of 0.9 ml/min was found to give acceptable chromatography for BDP on a Apollo C_{18} 5 µm, 150 mm x 4.6 mm column (alltech associates, deerfield, IL, USA). Ultraviolet detection was done at 240 nm and the retention time of BDP was 2.7 min. The on-line HPLC method was characterized to be accurate, precise, sensitive, and specific.

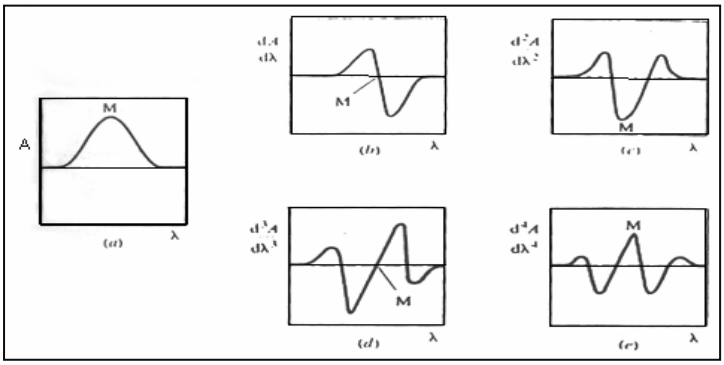
8. **Quantitative, highly sensitive liquid chromatography–tandem mass spectrometry method for detection of synthetic corticosteroids.**[36]

Stable isotopes of cortisol-9, 11, 12, 12-d_4 and triamcinolone-d_1 acetonide-d_6 were added as internal standards to calibrators, controls, and unknown samples. After acetonitrile precipitation, these samples were extracted with methylene chloride, and the extracts were washed and dried. Reconstituted extract (15 µl) was injected on a reversed-phase column and analyzed by LC-MS/MS in positive-ion mode.

METHOD: FIRST ORDER DERIVATIVE METHOD.[37, 38]

For the purpose of spectral analysis in order to relate chemical structure to electronic transitions and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy. Derivative Spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. (**Fig. No. 4.2**).

Fig: 4.2. Zero, first, second, third and fourth order derivative spectra.



In the context of derivative Spectrophotometry, the normal absorption spectrum is referred to as the fundamental/ zero order/ D^0 spectrum.

The first derivative D^1 spectrum is a plot of the rate of change of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ against λ. The maximum positive and maximum negative slopes respectively in the D^0 spectrum corresponds with a maximum and a minimum respectively in the D^1 spectrum. The λ_{max} in D^1 spectrum is a wavelength of zero slope and gives $dA/ d\lambda = 0$ in the D^1 spectrum.

The second derivative D^2 spectrum is a plot of the curvature of the D^0 spectrum against wavelength or a plot of $d^2A/d\lambda^2$ against λ. The maximum negative curvature in the D^0 spectrum gives a minimum in the D^2 spectrum, and the maximum positive curvature in the D^0 spectrum gives two small maxima called satellite bands in the D^2 spectrum. The wavelength of maximum slope and zero curvature in the D^0 spectrum correspond with cross-over points in the D^2 spectrum.

These spectral transformations confer two principal advantages on derivative Spectrophotometry.

- An even order spectrum is of narrower spectral bandwidth than its fundamental spectrum. A derivative spectrum therefore shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the λ_{max} of the individual bands.
- Derivative Spectrophotometry discriminates in favor of substances of narrow spectral bandwidth against broad bandwidth substances. All the amplitudes in the derivative spectrum are proportional to the concentration of the analyte, provided that Beer's law is obeyed by the fundamental spectrum.

The enhanced resolution and bandwidth discrimination increases with increasing derivative order. However, it is also found that the concomitant increase in electronic noise inherent in the generation of the higher order spectra, and the consequent reduction of the signal-to-noise ratio, place serious practical limitations on the higher order spectra. For quantitative purpose, second and fourth derivative spectra are the most frequently employed derivative order.

Derivative spectra may be generated by any of three techniques. The earliest derivative spectra were obtained by modification of the optical system. Spectrophotometers with dual monochromators set a small wavelength interval ($\Delta\lambda$, typically 1-3 nm) apart, or with the facility to oscillate the wavelength over a small range, are required. In either case the photo detector generates a signal with amplitude proportional to the slope of the spectrum over the wavelength interval.

The second technique to generate derivative spectra is electronic differentiation of the spectrophotometer analog signal. Resistance capacitance (RC) modules may be incorporated in series between the spectrophotometer and recorder to provide differentiation of the absorbance, not with respect to wavelength, but with respect to time, thereby producing the signal dA/dt. If the wavelength scan rate is constant ($d\lambda/dt = C$), the derivative with respect to wavelength is given by

$$dA/d\lambda = (dA/dt) / (d\lambda/dt) = (dA/dt)(1/C)$$

Derivative spectra obtained by RC modules are highly dependent on instrumental parameters, in particular the scan speed and the time constant. It is essential, therefore, to employ a standard solution of the analyte to calibrate the measured value under the instrumental conditions selected. The third technique is based upon microcomputer differentiation. Microcomputers incorporated into or interfaced with the spectrophotometer may be programmed to provide derivative spectra during or after the scan, to measure derivative amplitudes between specified wavelengths and to calculate concentrations and associated statistics from the measured amplitude.

EXPERIMENTAL

A. Preparation of standard stock solutions and selection of wavelengths and analytical concentrations.

1. Solvent:

Methanol AR grade is used as solvent.

2. Preparation of standard stock solutions:

50 mg each of Salbutamol Sulphate and Beclomethasone Dipropionate were weighed separately and transferred into two different 50 ml volumetric flasks. Both the drugs were dissolved in 25 ml of methanol by ultrasonication and then volume was made up to the mark with methanol to obtain final concentration of 1000 µg/ml of each component (stock 'A' solution). From the above stock 'A' solution, 10 ml of aliquots were pipetted out in a 100 ml volumetric flask separately and the volume was made up to the mark with methanol to obtain the final concentration of 100 µg/ml of each component (stock 'B' solution).

3. Selection of wavelengths:

By dilution of stock B of each drug, 10 µg/ml solution of Salbutamol Sulphate and Beclomethasone Dipropionate were prepared and both the solutions were scanned separately in the wavelength region of 400 – 200 nm. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected are such that at each wavelength the absorbance difference between the components is as large as possible. From the examination of the first derivative spectra of Beclomethasone Dipropionate and Salbutamol Sulphate, 278.5 nm (λ_1) and 239.5 nm (λ_2) were selected as working wavelengths for the first order derivative spectroscopy, as at 278.5 nm SS exhibited zero absorbance and at 239.5 nm BDP exhibited zero absorbance.

4. **Selection of analytical concentrations**

❖ **Beclomethasone Dipropionate**:

Appropriate aliquots were pipetted out from the standard stocks 'A' and 'B' solutions into a series of 10 ml volumetric flasks. The volume was made upto the mark with methanol to get a set of solutions having the concentrations ranging from 1 to 150 µg/ml. Absorbance of the above solutions were measured at 278.5 nm (zero crossing of SS), and a calibration curve of absorbance against concentration was plotted.

❖ **Salbutamol Sulphate**:

Appropriate aliquots were pipetted out from the standard stocks 'A' and 'B' solutions into a series of 10 ml volumetric flasks. The volume was made upto the mark with methanol to get a set of solutions having the concentrations ranging from 1 – 200 µg/ml. Absorbance of the above solutions were measured at 239.5 nm (zero crossing of BDP), and a calibration curve of absorbance against concentration was plotted.

B. Analysis of pure drug mixture.

10 mg of standard Beclomethasone Dipropionate and 20 mg of standard Salbutamol Sulphate were weighed separately and transferred in a 100 ml volumetric flask. The drug mixture was allowed to dissolve in sufficient quantity of methanol by ultrasonication and then volume was made up to the mark with methanol to obtain a mixture with final concentration of 100 µg/ml of Beclomethasone Dipropionate & 200 µg/ml of Salbutamol Sulphate (stock 'A' solution). From the above stock solution 'A', 2 ml of the aliquot was pipetted out and was transferred to a 10 ml volumetric flask. The volume was made up to 10 ml with methanol to obtain a solution with final concentration of Beclomethasone Dipropionate and Salbutamol Sulphate, 20 µg/ml & 40 µg/ml respectively. Six replicates were prepared and analyzed at the selected analytical wavelengths, 239.5 nm and 278.5 nm.

C. Analysis of formulation.

Dissolve 20 capsules with 50 ml of methanol in 50 ml volumetric flask. This gives 160 µg/ml of Salbutamol Sulphate and 80 µg/ml of Beclomethasone Dipropionate. Take 10 ml and filter by using Whatman filter paper No.41. From the filtered portion take 3.625 ml and dilute to 10 ml with methanol. This gives 58 µg/ml of Salbutamol Sulphate and 29 µg/ml of Beclomethasone Dipropionate respectively. The sample solutions were analyzed at 278.5 nm and 239.5 nm and concentrations are obtained from the regression equations.

METHOD VALIDATION.[17]

A. ACCURACY:

Application of the analytical procedure to synthetic mixtures of the drug product components to which 80 %, 100 %, 120 % of the drug substance to be analyzed have been added.

❖ **80 % level of recovery:**

Dissolve 20 capsules in 25 ml of methanol in 50 ml volumetric flask add 6.4 mg and 3.2 mg of Salbutamol Sulphate, and Beclomethasone

Dipropionate respectively. Subject for ultrasonication and make up the volume with methanol. This gives 288 µg/ml of Salbutamol Sulphate and 144 µg/ml of Beclomethasone Dipropionate. Take 10 ml from this solution and filter by using Whatman filter paper No.41. From the filtered portion take 1 ml and dilute to 10 ml with methanol. This gives 28.8 µg/ml of Salbutamol Sulphate and 14.4 µg/ml of Beclomethasone Dipropionate respectively.

❖ **100 % and 120 % level of recovery:**

These solutions are prepared in the similar way as in 80 % level of recovery.

Table 4.1.Amount of BDP and SS added at different levels of accuracy.

% level of recovery	No of capsules taken	Amount of SS added(mg)	Amount of BDP added(mg)	Conc. of SS (µg/ml)	Conc. of BDP (µg/ml)
80	20	6.4	3.2	28.8	14.4
100	20	8	4	32	16
120	20	9.6	4.8	35.2	17.6

The mixed sample solutions were analyzed by measuring their absorbance values. The concentration of Beclomethasone Dipropionate and Salbutamol Sulphate were calculated using simultaneous equations. At each level of recovery studies, three determinations were performed at 239.5 nm and 278.5 nm. The absorbance values are substituted in the regression equations and the concentrations are calculated.

PRECISION:

Precision is carried at 2 levels: repeatability, and intermediate precision.

❖ **Repeatability:**

Six determinations at 100 % of test concentration i.e. 200 µg/ml and 150 µg/ml of Salbutamol Sulphate and Beclomethasone Dipropionate respectively. Analysis is carried on mixed standard solutions. Six replicates were prepared and analysed at 278.5 nm and 239.5 nm. The results are validated statistically.

❖ **Intermediate precision:**

Carried on 3 days by 3 analysts using a set of six sample mixtures containing 25 µg/ml of Beclomethasone Dipropionate and 50 µg/ml of Salbutamol Sulphate. Solutions were prepared and analyzed at same time on different days at their selected analytical wavelengths 278.5 nm & 239.5 nm. The variation of the results on different days was analyzed and statistically validated.

B. SPECIFICITY:

It involved demonstration of the discrimination of the analyte in the presence of excipients. This is done by spiking pure substances with appropriate levels of excipients and demonstrating that the assay result is unaffected by the presence of that material.

It involves 2 steps

❖ **First step:**

Preparation of a set of six mixed standard solutions containing 25 µg/ml and 50 µg/ml of Beclomethasone Dipropionate and Salbutamol Sulphate respectively and analyzing.

❖ **Second step:**

Addition of 5 mg of excipients to 10 ml of each of the solutions in first step and subject for ultrasonication, followed by filtration. Filterate is used for analyzing.

C. RANGE:

Range is tested from 50 % to 120 % of test concentration. Mixed standard solutions were prepared and analyzed. 3 replicates for each concentration are prepared. The concentration of Beclomethasone Dipropionate and Salbutamol Sulphate at different % of concentration are clearly given in table

Concentrations of BDP and SS at different % of concentration.

Compound name	50%	100%	105%	120%
BDP	75	150	157.5	180
SS	100	200	210	240

Note: The concentrations are in µg/ml.

METHOD: FIRST ORDER DERIVATIVE METHOD

Fig: 5.7. First order derivative spectra of BDP.

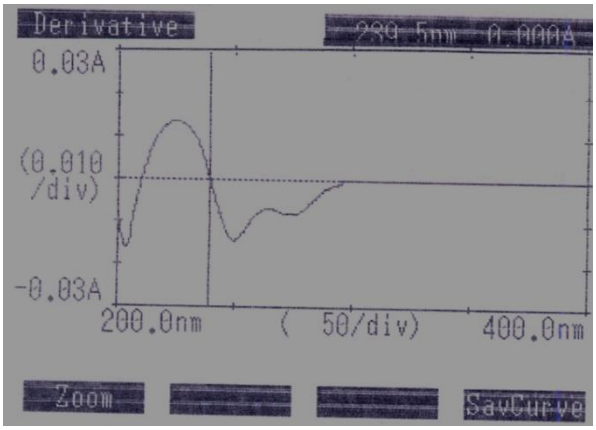


Fig: 5.8. First order derivative spectra of SS.

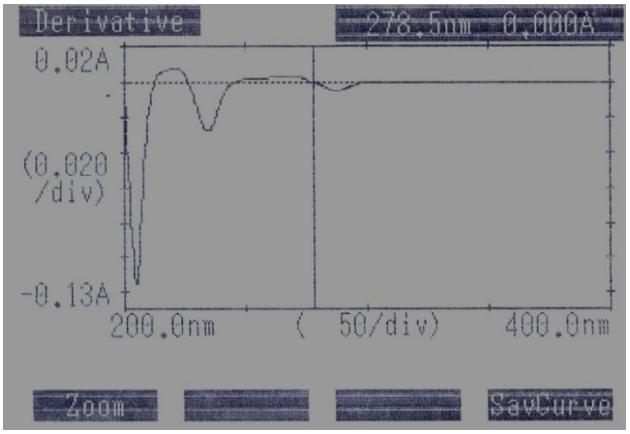


Fig: 5.9. First order derivative spectra of mixture at 239.5 nm.

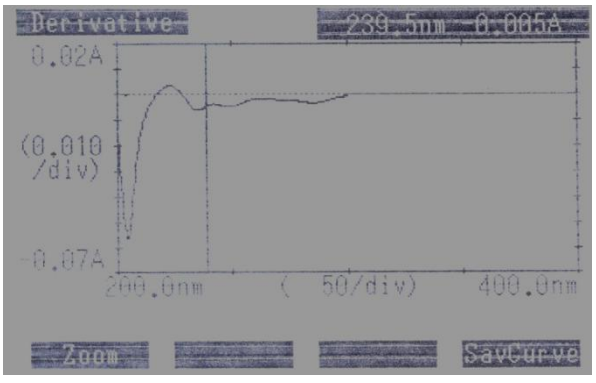


Fig: 5.10. First order derivative spectra of mixture at 278.5 nm.

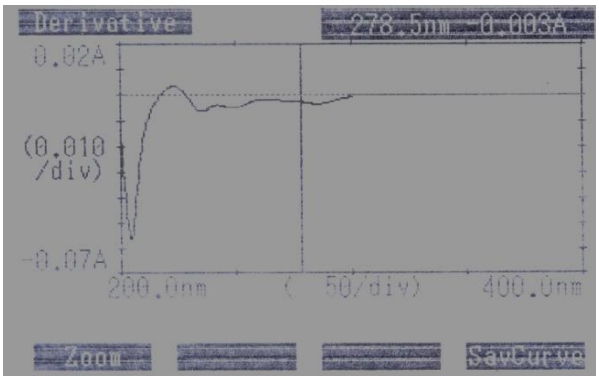


Table: 5.20. Result of calibration curve for BDP at 278.5 nm.

Concentration (µg/ml)	Absorbance Mean (n=6)
1	-0.002
10	-0.007
20	-0.012
30	-0.016
40	-0.024
50	-0.027
60	-0.032
70	-0.037
80	-0.045
90	-0.049
100	-0.056
110	-0.061
120	-0.065
130	-0.069
140	-0.074
150	-0.081

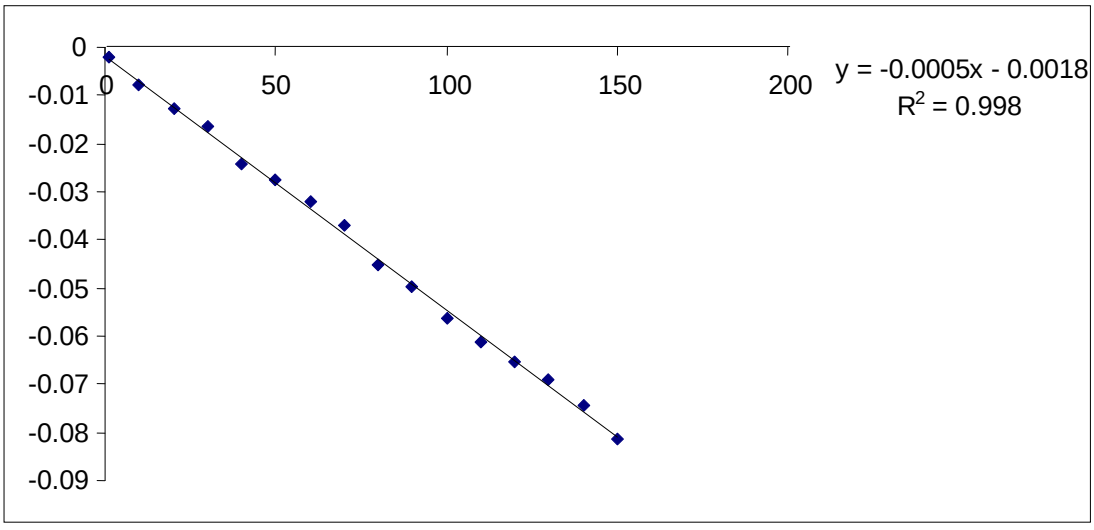


Fig: 5.11. Calibration curve for BDP at 278.5 nm.

Table: 5.21. Result of calibration curve for SS at 239.5 nm.

Concentration (µg/ml)	Absorbance Mean (n=6)
1	**-0.002**
10	**-0.015**
20	**-0.026**
30	**-0.042**
40	**-0.054**
50	**-0.067**
60	**-0.082**
70	**-0.093**
80	**-0.106**
90	**-0.123**
100	**-0.130**
110	**-0.145**
120	**-0.157**
130	**-0.174**
140	**-0.183**
150	**-0.2**
160	**-0.211**
170	**-0.221**
180	**-0.233**
190	**-0.246**
200	**-0.256**

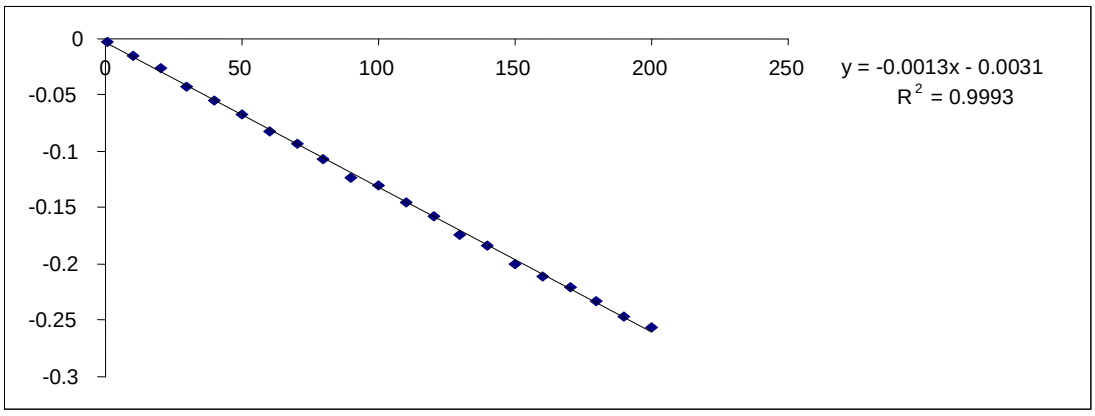


Fig: 5.12. Calibration curve for SS at 239.5 nm.

Table: 5.22. Data of BDP and SS at 278.5 nm and 239.5 nm.

Parameter	BDP at 278.5 nm	SS at 239.5 nm
Linearity (µg/ml)	1-150	**1-200**
Slope	-0.0005	**-0.0013**
Intercept	**-0.0018**	**-0.0031**

Table: 5.23. Assay results of standard drug mixture.

Sl. No.	Amount present in (µg/ml)		Amount obtained in (µg/ml)		Amount obtained in (%)	
	BDP	**SS**	**BDP**	**SS**	**BDP**	**SS**
1	20	40	19.669	39.862	98.346	99.657
2	20	40	19.955	39.489	99.777	98.724
3	20	40	19.760	39.294	98.804	98.236
4	20	40	19.898	40.191	99.491	100.478
5	20	40	19.769	39.307	98.849	98.269
6	20	40	19.685	39.85	98.427	99.625

- **The concentration of BDP and SS is 98.949 µg/ml and 99.164 µg/ml.**

Table: 5.24. Assay results of formulation.

Sl. No.	Amount present in (µg/cap)		Amount obtained in (µg/cap)		Label Claim (%)	
	BDP	**SS**	**BDP**	**SS**	**BDP**	**SS**
1	200	400	198.084	390.892	99.042	97.723
2	200	400	196.486	395.264	98.243	98.816
3	200	400	200.594	397.376	100.297	99.344
4	200	400	196.922	394.112	98.461	98.528
5	200	400	196.086	397.104	98.043	99.276
6	200	400	198.718	394.472	99.359	98.618

- **The concentration of BDP and SS is 98.907 µg/ml and 98.717 µg/ml.**

Table: 5.25. Determination of accuracy of BDP and SS.

Level of % recovery	Amount present (µg/20 CAP)		Amount of standard drug added (µg)		Total amount recovered (µg)		% Recovery	
	BDP	**SS**	**BDP**	**SS**	**BDP**	**SS**	**BDP**	**SS**
80%	4000	8000	3200	6400	6999.696	14147.14	97.218	98.244
	4000	8000	3200	6400	7077.312	14303.23	98.296	99.328
	4000	8000	3200	6400	7218.936	14125.54	100.263	98.094
100%	4000	8000	4000	8000	7951.92	15700.96	99.399	98.131
	4000	8000	4000	8000	7910.72	15731.84	98.884	98.324
	4000	8000	4000	8000	7887.2	16042.08	98.590	100.263
120%	4000	8000	4800	9600	8682.784	17677.79	98.668	100.442
	4000	8000	4800	9600	8558.528	17277.39	97.256	98.167

4000	8000	4800	9600	8818.744	17311.01	100.213	98.358

Table: 5.26. The % recovery of BDP and SS.

Components	80%	100%	120%
BDP	98.592	98.957	98.712
SS	98.555	98.906	98.989

Table: 5.27. Repeatability data for BDP.

Sl.No	Amount of BDP present (µg/ml)	Amount of BDP obtained (µg/ml)	Label claim (%)
1	150	148.821	99.214
2	150	149.284	99.523
3	150	150.546	100.364
4	150	149.379	99.586
5	150	148.288	98.859
6	150	149.067	99.378

Table: 5.28. Repeatability data for SS.

Sl.No	Amount of SS present (µg/ml)	Amount of SS obtained (µg/ml)	Label claim (%)
1	200	198.568	99.284
2	200	199.389	99.694
3	200	200.764	100.382
4	200	198.982	99.491
5	200	199.853	99.926
6	200	200.247	100.123

Table: 5.29. Statistical validation data for repeatability.

Components	Mean*	Standard deviation*	Co-efficient of variation*
BDP	99.487	0.502	0.504
SS	99.816	0.407	0.408

***n = 6**

Table: 5.30. Determination of intermediate precision of SS and BDP.

Sl. No.	Amount present in (µg/ml)		Amount obtained in (µg/ml)		Label Claim (%)	
	BDP	**SS**	**BDP**	**SS**	**BDP**	**SS**
DAY – I, ANALYST-I						
1	25	50	25.6	50.411	102.400	100.822
2	25	50	25.474	49.711	101.896	99.423
3	25	50	25.282	50.699	101.128	101.399
4	25	50	25.590	49.953	102.362	99.907
5	25	50	25.382	50.345	101.528	100.690
6	25	50	25.219	49.959	100.878	99.919
DAY – II, ANALYST-II						
1	25	50	25.122	49.588	100.488	99.177
2	25	50	25.393	50.196	101.572	100.392
3	25	50	25.663	50.514	102.652	101.028
4	25	50	25.054	49.955	100.219	99.910
5	25	50	25.410	50.663	101.643	101.327
6	25	50	25.591	50.374	102.364	100.749
DAY – III, ANALYST-III						
1	25	50	25.215	50.146	100.862	100.292
2	25	50	25.331	50.993	101.324	101.986
3	25	50	25.379	49.698	101.519	99.397
4	25	50	25.519	49.588	102.076	99.177
5	25	50	25.545	50.962	102.18	101.924
6	25	50	25.357	50.475	101.428	100.951

Table: 5.31. Statistical validation data for intermediate precision.

Components	Mean*	Standard deviation*	Co-efficient of variation*
BDP	101.584	0.690	0.679
SS	100.470	0.886	0.882

***n = 18**

Table: 5.32. Data of BDP and SS before spiking with excipients.

Sl.No.	Amount present (µg/ml)		Amount obtained (µg/ml)		Label Claim (%)	
	BDP	**SS**	**BDP**	**SS**	**BDP**	**SS**
1	25	50	24.706	49.887	98.824	99.774
2	25	50	24.664	49.619	98.658	99.238
3	25	50	24.543	49.658	98.173	99.316
4	25	50	24.711	49.699	98.846	99.398
5	25	50	24.661	49.658	98.645	99.316

6	25	50	24.685	49.715	98.742	99.425

Table: 5.33. Data of BDP and SS after spiking with excipients.

Sl.No.	Amount present (µg/ml)		Amount obtained (µg/ml)		Label claim (%)	
	BDP	**SS**	**BDP**	**SS**	**BDP**	**SS**
1	25	50	24.656	49.771	98.625	99.543
2	25	50	24.635	49.591	98.543	99.183
3	25	50	24.519	49.584	98.078	99.168
4	25	50	24.660	49.606	98.642	99.213
5	25	50	24.608	49.611	98.432	99.222
6	25	50	24.569	49.573	98.279	99.147

Table: 5.34. Effect of excipients on the method.

Components	% of conc. before spike	% of conc. after spike	Difference in the % of conc.
BDP	98.648	98.433	0.215
SS	99.411	99.246	0.165

- **The concentration of the drugs are unaffected by the addition of excipients.**

Table: 5.35. The range of BDP.

Sl.No	% level of conc.	Amount present (µg/ml)	Amount obtained (µg/ml)	Label claim (%)
1	50	75	75.071	100.095
2	100	150	150.037	100.025
3	105	157.5	176.829	112.273
4	120	180	163.353	90.752

Table: 5.36. The range of SS.

Sl.No	% level of conc.	Amount present (µg/ml)	Amount obtained (µg/ml)	Label claim (%)
1	50	100	98.272	98.272
2	100	200	200..51	100.255
3	105	210	358.827	170.87
4	120	240	121.108	50.462

- **The range is only upto 100 % of test concentration for BDP (150 µg/ml) and SS (200 µg/ml).**

FIRST ORDER DERIVATIVE:

This method is good interms of accuracy, precision (repeatability, intermediate precision), specificity and analysis of marketed formulation. Interferences are eliminated by conversion to first order. Samples are analyzed simultaneously without any separation. This method is simple.

REFERENCES

1. http://www.springerlink.com/content/notcqjy429mljcbo/
2. http://www.springerlink.com/content/m80m185g1526k435/
3. Connors KA. A Textbook of Pharmaceutical Analysis, 3rd ed. Delhi: Wiley intersciences Inc.; 1994.
4. http://www.pharmainfo.net/reviews/methods-estimation-multicomponent-formulations-review.
5. Parimoo P. Pharmaceutical Analysis, CBS Publishers and Distributors, New Delhi, 145.

6. Mendham J, Denny RC, Thomas M, Vogel's Text Book of Quantitative Analysis, 6th ed, Pearson Education Limited, 2004, 1.
7. Drug index. New delhi: Passi; 2007; 11(1): 432,425.
8. Indian Pharmacopoeia, The Controller of Publication, New Delhi, Vol-3, 2007,144,153, 1063-64, vol-2, 2007, 154-55.
9. http://www.sciencelab.com/XMSDS-Albuterol_sulphate-9922819
10. O Neil MJ. The Merck index-an encyclopedia of chemicals, Drugs and biologicals. 13th ed. New Jersey: merck & Co; 2001.
11. http://www.sciencelab.com/XMSDS-Beclomethasone_dipropionate_micronized_U_S_P-9923030
12. http://www.pharmainfo.net/reviews/various-UV-spectrophometric-simultaneous-estimation-methods.
13. http://chromatographonline.findanalytichem.com/lcgc/data/articlestandard/lcgc/242005/164646/article.pdf
14. http://www.chem.agilent.com/enus/products/instruments/lc/1200seriesrapidresolutionlcsystem/pages/default.aspx
15. http://gmpua.com/validation/method/AnalyticalChem/AnalyticalChemistry.htm.
16. http://www.39hg.com/jp14e/14dataValidation_of_Analytical_pr.pdf.
17. http://www.ich.org/LOB/media/MEDIA417.pdf
18. http://www.lcresources.com/resources/TSWiz/hs150.htm
19. Koh YM, Saleh MI, Tan SC. Selective extraction of salbutamol from human plasma with the use of phenylboronic acid. J. Chromatogr. A 2003; 987: 257-267.
20. Adrian fesser CE, Dickson LC, Macneil AD, John RP, Stephen L, Ronald G. Determination of β-Agonists in Liver and Retina by Liquid Chromatography-Tandem Mass spectrometry. J. AOAC. Int 2005; 88: 61-69.
21. Bernal JL, Del Nozal MJ, Velasco H, Toribio L. HPLC versus SFC for the determination of salbutamol sulphate and its impurities in pharmaceuticals. J. liq chromatogr. Related tech 1996; 19: 1579-1590.
22. Joseph Lau HW, Cheang SK, John EM. Determination of Clenbuterol, Salbutamol, and Cimaterol in Bovine Retina by Electrospray Ionization–Liquid Chromatography–Tandem Mass Spectrometry. J. AOAC. Int 2004; 87: 31-38.
23. Zhang XZ, Gan YR, Zhao FN. Determination of Salbutamol in Human Plasma and Urine by High-Performance Liquid Chromatography with a Coulometric Electrode Array System. J Chromatoger Sci, 2004; 42: 263-267.
24. Black SB, Hansson RC. Determination of Salbutamol and Detection of Other β Agonists in Human Postmortem Whole Blood and Urine by GC–MS-SIM. J Anal Toxicol 1999; 23: 113-118.
25. Colthup PV, Dallas FA, Saynor DA, Carey PF, Skidmore LF, Martin LE, Wilson K. Determination of salbutamol in human plasma and urine by high-performance thin-layer chromatography. J Chromatogr 1985; 345(1): 111-118.
26. Aboulenein HY, Mariana S. Analysis of Salbutamol and Related Impurities by Derivative Spectrometry. Arch Pharm 2000; 133(4):75-78.
27. Lúcia D, Rosa , Jordi O, Jordi S .Derivatization procedures for the detection of β2 agonists by gas chromatography/mass spectrometric analysis. J Mass Spectrom 2000; 35(11): 1285 – 1294.
28. Colthup PV, Dallas FA, Saynor DA, Carey PF, Skidmore LF, Martin LE, Wilson K. Determination of salbutamol in syrups by capillary electrophoresis with contact less conductivity detection, J Pharm Biomed Anal 2006; 40(5): 1288-1292.
29. Girault J, Istin B, Malgouyat J M, Brisson A M, Fourtillan J B .Simultaneous determination of beclomethasone, beclomethasone monopropionate and beclomethasone, dipropionate in biological fluids using a particle beam interface for combining liquid chromatography with negative-ion chemical ionization mass spectrometry. J Chromatogr 1991; 564: 43-53.
30. Batavia R, Taylor KM, Craig DQ, Thomas M. The measurement of beclomethasone dipropionate entrapment in liposomes: a comparison of a microscope and an HPLC method. Int J Pharm 2001; 212(1):109-19.
31. Deventer K, Van Eenoo P, Delbeke F T. Screening for anabolic steroids in doping analysis by liquid chromatography/electrospray ion trap mass spectrometry. Biomed Chromatogr 2005; 20(5): 429 – 433.
32. Deorsi D, Gagliardi L, Chimenti F, Tonelli D. HPLC determination of beclomethasone dipropionate and its degradation products in bulk drug and pharmaceutical formulations. Anal Lett 1995; 28: 1655 – 1663.
33. Dewasch K, Debrabander T, Courtheyn D, Vanpeteghem C. Detection of corticosteroids in injection sites and cocktails by MS^n. Analyst 1998; 123: 2415 – 2422.
34. Dirk C, Jan Vercammen, Maureen L, Hilde S, Katia DW, Hubert DB. LC/MS-MS method for the determination of Beclomethasone Dipropionate and Beclomethasone-17-monoproprionate in human plasma. Analyst 1998; 123: 2409 – 2414.
35. Gupta A, Myrdal PB. On-line High-performance liquid chromatography method for analyte quantitation from pressurized metered dose inhalers. J Chromatogr 2004; 1033(1):101-106.
36. Robert LT, Stefan KG, Ravinder J**S.** Quantitative**,** Highly Sensitive Liquid Chromatography–Tandem Mass Spectrometry Method for detection of synthetic corticosteroids. Clinchem journal 2004; 10:1373**.**
37. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. Delhi: CBS Publishers and Distributors; 1997.
38. Instruction Manual Operation Guide UV-1700, Shimadzu Spectrophotometer, Shimadzu Corporation, Kyoto, Japan.

***Corresponding Author:**
Venkata naveen.T*
Dept. of Pharmaceutical Analysis,
National College of Pharmacy,
Shimoga