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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SALMETEROL AND FLUTICASONE PROPIONATE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A new method was established for simultaneous estimation of Salmeterol and Fluticasone propionate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Salmeterol and Fluticasone propionate by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer (KH2PO4and K2HPO4) phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.170 mins and 7.280mins. The % purity of Salmeterol and Fluticasone propionate was found to be 99.1% and 98.2% respectively. The system suitability parameters for Salmeterol and Fluticasone propionate such as theoretical plates and tailing factor were found to be 12294, 1.27 and 10491 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Salmeterol and Fluticasone propionate was found in concentration range of 16µg-80µg and 25µg-125µg and correlation coefficient (r2) was found to be 0.999 and 0.998, % recovery was found to be 101.7% and 102.0%, %RSD for repeatability was 0.8and 0.5, % RSD for intermediate precision was 1.99 and 1.82 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

KEY WORDS: Salmeterol and Fluticasone propionate, C18 column, RP-HPLC.

INTRODUCTION

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity.

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The development of new chem- ical entities (NCEs) is comprised of two major activities: drug discovery and drug development. The goal of the drug discovery program is to investigate a plethora of compounds employing fast screening approaches, leading to gen- eration of lead compounds and then narrowing the selection through targeted synthesis and selective screening (lead optimization). This lead to the final selection of the most potentially viable therapeutic candidates that are taken forward to drug development. The main functions of drug development are to completely characterize candidate compounds by performing drug metabolism, preclinical and clinical screening, and clinical trials. Concomitantly with the drug development process, the optimization of drug synthesis and formulation are performed which eventually lead to a sound and robust manufacturing process for the active pharmaceutical ingredient and drug product. Throughout this drug discovery and drug development paradigm, rugged analytical HPLC separation methods are developed and are tailored by each development group (i.e., early drug discovery, drug metabolism, pharmokinetics, process

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research, preformulation, and formulation). At each phase of development the analyses of a myriad of samples are performed to adequately control and monitor the quality of the prospective drug candidates, excipients, and final products Effective and fast method development is of paramount importance throughout this drug development life cycle. This requires a thorough understanding of HPLC principles and theory which lay a solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization.

In RP-HPLC the stationary phase is non-polar often a hydrocarbon and the mobile phase is relatively polar such as water, methanol or Acetonitrile. In RPC the solutes are eluted in the order of their decreasing polarities. These are prepared by treating the surface of silanol group with an organochlorosilane reagent.

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface.

Salmeterol is a long-acting beta-2 adrenergic receptor agonist drug that is currently prescribed for the treatment of asthma and chronic obstructive pulmonary disease COPD. It has a longer duration of action than the short-acting beta-2 adrenergic receptor agonist, salbutamol.5 Salmeterol was first described in the literature in 1988.6 Salmeterol's structure is similar to salbutamol's with an aralkyloxy-alkyl substitution on the amine. Salmeterol is a long acting beta-2 adrenergic receptor agonist that binds to both the active and exo sites of the beta-2 adrenergic receptor.1 Salmeterol has a longer duration of action than other beta-2 agonists like salbutamol.5 Patients should be counselled regarding the risks of long acting beta agonist (LABA) monotherapy, hypokalemia, hypoglycemia, and not to take this drug with another LABA.

Fluticasone propionate is a synthetic glucocorticoid9,10Label. These drugs are available as inhalers, nasal, sprays, and topical treatments for various inflammatory indications9,10Label. Fluticasone propionate was first approved in 1990. Fluticasone propionate works through an unknown mechanism to affect the action of various cell types and mediators of inflammation10. Fluticasone propionate activates glucocorticoid receptors and inhibits lung eosinophilia in rats.

MATERIALS & METHODS

Chemicals and standards used

Table 1: List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
	Water	Merck	HPLC grade
	Methanol	Merck	HPLC grade
	Acetonitrile	Merck	HPLC grade
	Ortho phosphoric acid	Merck	G.R
	KH_2PO_4	Merck	G.R
	K_2HPO_4	Merck	G.R
	0. 22µ Nylon filter	Advanced lab	HPLC grade
	0.45µ filter paper	Millipore	HPLC grade
	Salmeterol and Fluticasone propionate	In – House	In- House

Instruments used

Table 2: List of instruments used

S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	HPLC- Shimadzu	model No. SPD-20MA LC+20AD	Software- LC-20 Solution	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India
3	Digital weighing balance(sensitivity 5mg)	ER 200A	-	Ascoset

4	pH meter	AD 102U	-	ADWA
5	Sonicator	SE60US	-	Enertech

Method development

Chromatographic trials for simultaneous estimation of Salmeterol and Fluticasone propionate by RP- HPLC.

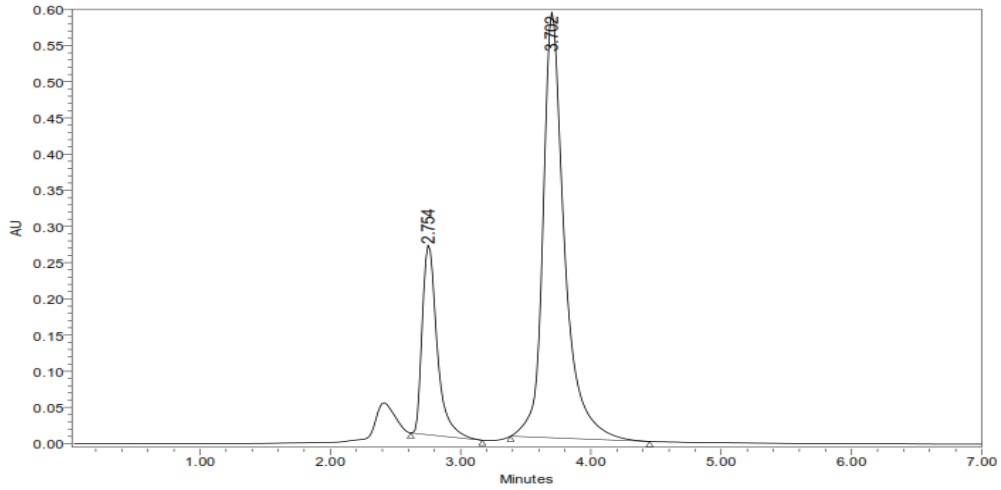
Trial-1

Chromatographic conditions

Column : Symmetry, C184.6x150mm, 5µm

Mobile phase ratio : MeOH:H_2O(50:50%v/v)
Detection wavelength : 236nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10min

Figure 1: Chromatogram showing trial 1 injection



Preparation of the Salmeterol and Fluticasone propionate standard and sample solution: Sample solution preparation: Accurately weigh and transfer 59.8 mg of Salmeterol and Fluticasone propionate Tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of Salmeterol and Fluticasone propionate the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents. Standard solution preparation:Accurately weigh and transfer 12.5 mg & 8 mg of Salmeterol and Fluticasone propionate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.6ml of Salmeterol and Fluticasone propionate the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. 10□L of the blank, standard and sample were injected into the chromatographic system and areas for the Salmeterol and Fluticasone propionate the peaks were used for calculating the % assay by using the formulae.

Assay calculation

Assay %=(sample area)/(Standard area)×(dilution sample)/(dilution of standard)×P/100×(Avg.wt)/Lc×100

Where:
Avg.wt = average weight of tablets
P= Percentage purity of working standard
Assay Results for Salmeterol =98.2%
Assay Results for Fluticasone propionate =99.1%

Validation parameters: Specificity, Linearity, Range, Accuracy, Precision, Repeatability, Intermediate Precision, Detection Limit, Quantitation Limit Preparation of stock solution: Accurately weigh and transfer 12.5 mg&8mg of Salmeterol and Fluticasone propionate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Preparation of Level – I (25ppmof Salmeterol &16ppm of Fluticasone propionate): 0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. Preparation of Level – II (50ppmof Salmeterol &32ppm of Fluticasone propionate):0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. Preparation of Level – III (75ppmof Salmeterol &48ppm of Fluticasone propionate): 0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. Preparation of Level – IV (100ppmof Salmeterol &64ppm of Fluticasone propionate):0.8ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (125ppmof Salmeterol &80ppm of Fluticasone propionate)1.0 of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

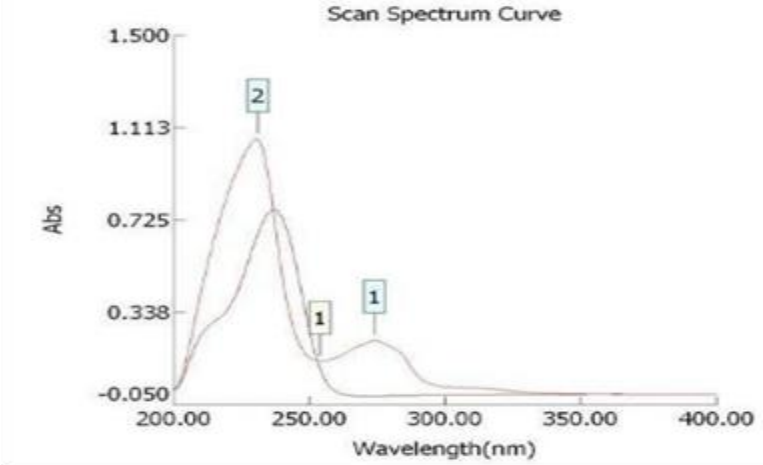
RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Salmeterol and Fluticasone propionate by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Salmeterol and Fluticasone propionate by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Salmeterol and Fluticasone propionate in pharmaceutical dosage form.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Salmeterol and Fluticasone propionate was obtained and the isobestic point of Salmeterol and Fluticasone propionate showed absorbance's maxima at 240 nm.

Figure 2: Spectrum showing overlapping spectrum of Salmeterol and Fluticasone propionate



The chromatographic method development for the simultaneous estimation of Salmeterol and Fluticasone propionate were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Salmeterol and Fluticasone propionate in API and pharmaceutical dosage form by RP-HPLC method.

Assay calculation for Salmeterol and Fluticasone propionate

The assay study was performed for the Salmeterol and Fluticasone propionate. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Fig. No.21-22 and results are tabulated in Table.NO.5.

Figure 3: Chromatogram showing assay of sample injection-1,2

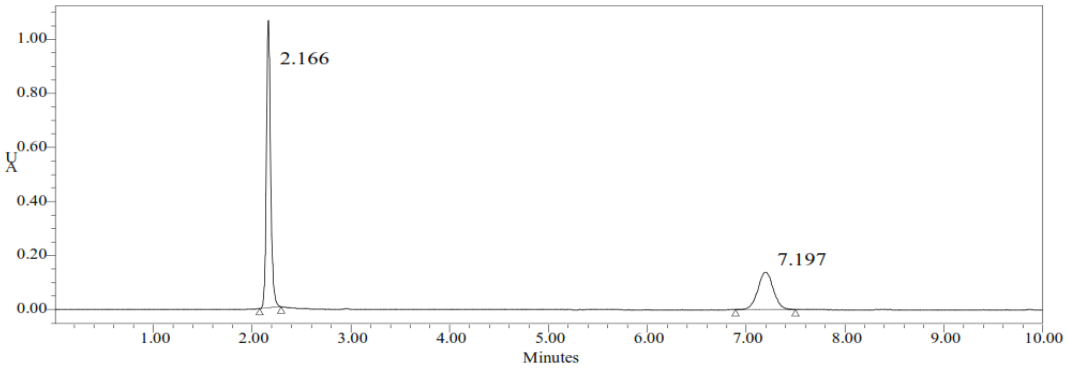


Figure 4: Chromatogram showing standard of sample injection.

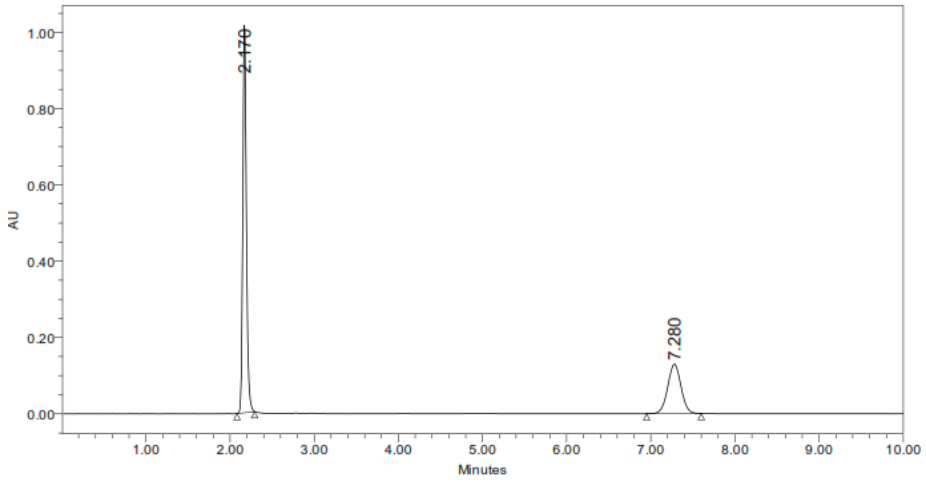


Table 3: Showing assay results

S. No	Name of compound	Label claim(mg)	Amount taken(mg)	%purity
1	Salmeterol	8	58.9	99.1
2	Fluticasone propionate	12.5	58.9	98.2

The retention time of Salmeterol and Fluticasone propionate was found to be 2.170 mins and 7.280 mins respectively. The system suitability parameters for Salmeterol and Fluticasone propionate such as theoretical plates and tailing factor were found to be 12458, 1.27and 10026, 1.03. Resolution was 8.67. The % purity Salmeterol and Fluticasone propionate in pharmaceutical dosage form was found to be 99.1 and 98.2% respectively.

Validation Report

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The specificity test was performed for Salmeterol and Fluticasone propionate. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity

The linearity study was performed for the concentration of 25 ppm to 150 for Salmeterol and 16ppm to 80ppm for Fluticasone propionate. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient.

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Salmeterol and Fluticasone propionate. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery the accuracy study was performed for % recovery of Salmeterol and Fluticasone propionate. The % recovery was found to be 101.4% and 101.7% respectively (NLT 98% and NMT 102%).

Precision

- Repeatability
- Intermediate Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision/Ruggedness

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Detection limit

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

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

Where

σ - Standard deviation (SD)

S - Slope

Figure 5: Results of LOD

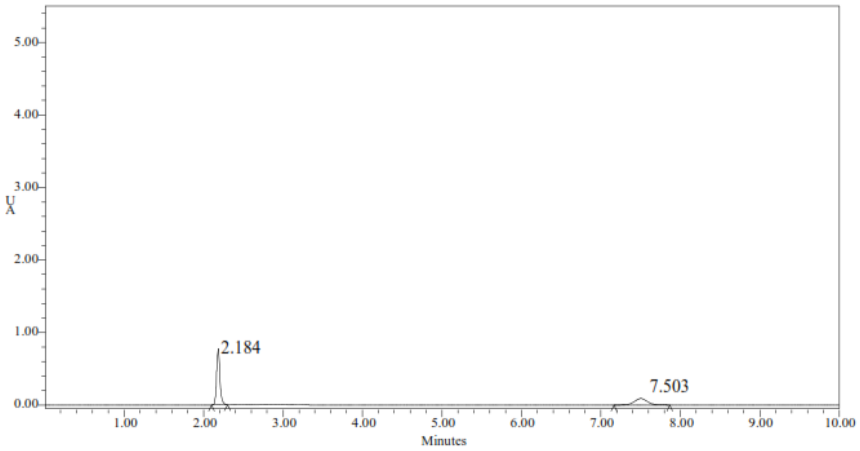


Table 4: Showing results for Limit of Detection

	Peak name	RT	Area	Height	USP PlateCount	USP Tailing
1	Salmeterol	2.184	430	154	12989.3	1.2
2	Fluticasone	7.503	1754	153	9945.7	1.0

The LOQ was performed for Salmeterol and Fluticasone propionate was found to be 6.60 and 0.112 respectively.

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Salmeterol and Fluticasone propionate. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

Figure 6: Chromatogram showing more flow rate 0.8ml/min

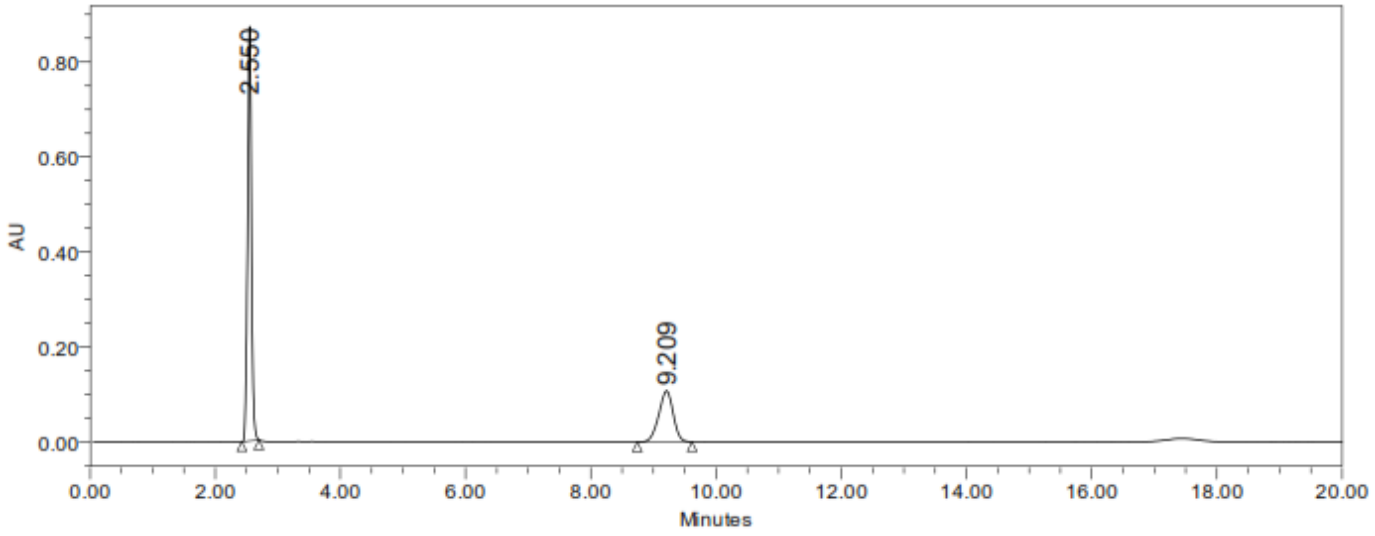
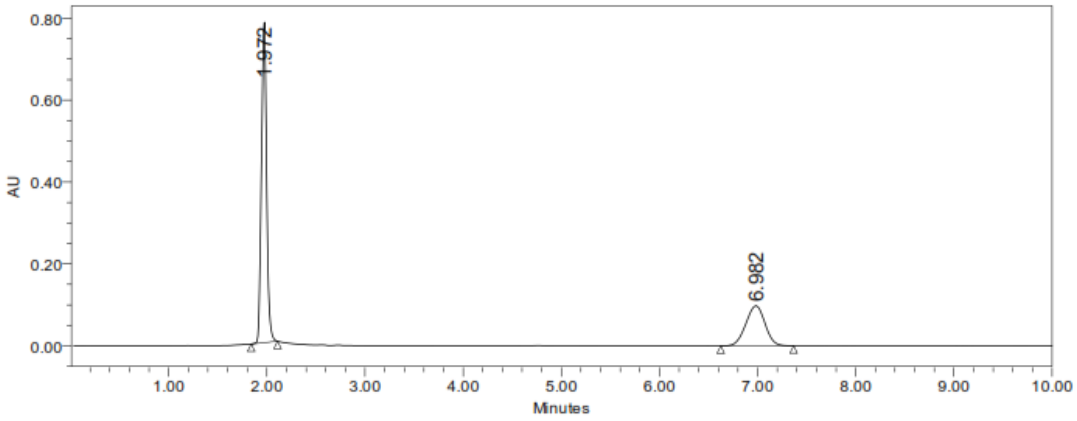


Figure 7: Chromatogram showing less flow rate 1.2 ml/min



The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

CONCLUSION

A new method was established for simultaneous estimation of Salmeterol and Fluticasone propionate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Salmeterol and Fluticasone propionate by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer (KH2PO4and K2HPO4) phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.170 mins and 7.280mins. The % purity of Salmeterol and Fluticasone propionate was found to be 99.1% and 98.2% respectively. The system suitability parameters for Salmeterol and Fluticasone propionate such as theoretical plates and tailing factor were found to be 12294, 1.27 and 10491 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Salmeterol and Fluticasone propionate was found in concentration range of 16µg-80µg and 25µg-125µg and correlation coefficient (r2) was found to be 0.999 and 0.998, % recovery was found to be 101.7% and 102.0%, %RSD for repeatability was 0.8and 0.5, % RSD for intermediate precision was 1.99 and 1.82 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Salmeterol and Fluticasone propionate in API and Pharmaceutical dosage form.

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