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Development and Validation of Uv Spectrophotometric and Rp-Hplc Method for the Estimation of Salbutamol Sulphate in Compressed Coated Tablet Dosage Form

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ABSTRACT
An accurate and reproducible UV spectrophotometric and RP-HPLC method for estimation of Salbutamol sulphate (SAL) was developed in the present work. The first developed method was UV spectrophotometric method, wavelength selected was 227 nm. Linearity was observed in concentration range of 4.8 to 24 µg/ml for salbutamol sulphate. Second developed method was RP-HPLC method using Kromstar C_{18} column (250 mm × 4.6 mm i.d.) and 10 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3.5 with orthophosphoric acid): Methanol: Acetonitrile in the ratio of 60: 30: 10% v/v/v as mobile phase. For RP-HPLC method, linearity was observed in the concentration range of 4.8-24 µg/ml for salbutamol Sulphate. Results of Validation of both analytical techniques were compiled as per ICH guideline Q2 (R1 & R2). The proposed analytical methods development and estimation of salbutamol sulphate and its validation by RP-HPLC is accurate, precise, simple, selective, sensitive, and rapid, hence this method can be effectively used for analysis of salbutamol sulphate in compressed coated tablets dosage form.

1. INTRODUCTION:

Salbutamol sulphate (SAL), chemically known as bis [(1RS)-2- [(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease (COPD) and it is official in Indian pharmacopoeia [1, 2] & British pharmacopoeia. Detailed survey of literature for SAL revealed several methods based on different techniques such as UV Spectro-photometry [3-7], RP-HPLC [8], and TLC [9] for its determination from various pharmaceutical dosage forms, but no any analytical method has been reported for compression coated tablets of SAL. Hence in the present work a successful attempt has been made to estimation of Salbutamol sulphate in compressed coated tablets dosage form by UV spectrophotometric method and RP-HPLC method. The proposed methods were optimized and validated as per ICH guideline Q2 (R1 & R2).

2. Experimental (Materials):

Salbutamol Sulphate was provided by Vamsi Laboratories, Flow Lac 100 (Lactose monohydrate) by DFE pharma, Klucel EXF (Hydroxypropyl cellulose) by Ashland Global, Colloidal Silicon dioxide (Aerosil 200) by Evonik, LIGAMED MF-2-V-MB (Magnesium Stearate) by Peter Greven , Methocel HPMC K100M DC2 (Hydroxy propyl methyl cellulose) by IIF, Methocel K15 M (Hydroxy propyl methyl cellulose) by IIF, A-tab (Dicalcium phosphate anhydrous) by Innophos and Milli Q Water (from in house lab) were used. Water, Acetonitrile and Methanol (HPLC Grade) for chromatography were procured from Finar chemicals Pvt. Ltd. All solutions were freshly prepared on daily basis.

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2.1 Instrumentation and analytical conditions:
The UV spectrophotometric method was performed on a Double-beam Shimadzu UV-Visible spectrophotometer, 1900, with UV probe software, spectral bandwidth of 1 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of drug solution. Working wavelength for UV method was 227 nm (λmax of SAL). RP-HPLC method was performed on HPLC system (Analytical manual Pvt. Ltd.) consisting of isocratic pump, column oven, and UV detector was employed for analysis. Chromatographic data was acquired using CTHX Analchrom software. Kromstar C_{18} column (4.6 mm i.d. × 250 mm) was used as stationary phase. SAL was eluted isocratically with a flow rate 1.0 ml/min using a mobile phase consisting of 10 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3.5 with orthophosphoric acid): Methanol: Acetonitrile in the ratio of 60: 30: 10% v/v/v as mobile phase, respectively. The wavelength of UV detector was set to 227 nm. The mobile phase was freshly prepared, filtered through 0.45 µm membrane filter (Millipore) and sonicated before use.

3. Preparation of solutions
3.1 Preparation of standard solutions
3.1.1 UV spectrophotometric method:
Standard stock solution containing SAL was prepared by dissolving quantity of Salbutamol sulphate equivalent to SAL base 10 mg in 100 ml of methanol in separate 100 ml volumetric flask and final volume of solution was made up to 100 ml with methanol to get stock solution containing each of 100 µg/ml of SAL. From these solutions, concentrations of 4.8 to 24 µg /ml were made in 10.0 ml volumetric flasks to take aliquots of 0.48, 0.96, 1.44, 1.92 and 2.4 ml from stock solution to get 4.8, 9.6, 14.4, 19.2, and 24 µg/ml of SAL.

3.1.2 RP-HPLC method:
Standard stock solution containing SAL was prepared by dissolving weighed quantity of Salbutamol sulphate equivalent to SAL base 10 mg in 100 ml of methanol in separate 100 ml volumetric flask and final volume of solution was made up to 100 ml with methanol to get stock solution containing each of 100 µg/ml of SAL. From these solutions, concentrations of 4.8-24 µg /ml were made in 10.0 ml volumetric flasks with mobile phase.

3.2 Preparation of the sample solutions
3.2.1 UV spectrophotometric method:
Twenty compressed coated tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablets powder equivalent to 9.6 mg of SAL was transferred to 100.0 ml volumetric flask; 1 ml was pipetted out in 10 ml vol. flask. The volume was then made up to the mark with methanol and sonicated for 10 min. The resulting solution was filtered through Whatman filter paper and filtrate was appropriately diluted to get approximate concentration of 9.6 µg/ml of SAL.

3.2.2 RP-HPLC method:
Twenty compressed coated tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablets powder equivalent to 9.6 mg of SAL was transferred to 100.0 ml volumetric flask and dissolved in mobile phase and sonicated for 20 min. and then volume was made up to the mark with mobile phase. The resulting solution was mixed and filtered through Whatman filter paper and 1 ml filtrate was appropriately diluted into 10 ml mobile phase to get approximate concentration of 9.6 µg/ml of SAL. The diluted solutions were filtered through 0.20 µm membrane filter to get clear solutions.

3.3 Preparation of compression coated tablets (CCTs)
The ancient process of compression coating was initially put forth by Noyes in a patent application in 1896. Using various grades of HPMC as the primary functional material to control the release rate, the goal of this work was to construct compression coated tablets containing salbutamol sulfate as active in core portion & control release polymers in outer mantle portion.

3.3.1 Preparation of the core tablets
The internal core portion and outer mantle or coat are the two key components of a compression coated tablets. A second turret with a larger die cavity is utilized to manufacture the compression coating for the core tablets, which is a tiny porous tablet that is prepared on one turret. Depending on the release mechanism, different release kinetics were achieved by preparing the inner-core tablets via wet granulation followed by compression. The wet granulation method was used to create the core tablets. Each tablet contained Salbutamol sulphate 9.60 mg, Flow Lac 100 (Lactose monohydrate) by DFE pharma 103.9mg as diluent, Klucel EXF (Hydroxypropyl cellulose) by Ashland Global 2 mg as binder. All the excipients were mixed properly and Klucel EXF (Hydroxypropyl cellulose) by Ashland Global with Milli Q water was added as binder. After forcing the wet mixture through a 20 mesh (ASTM) sieve, wet granules were created and dried for two hours at 70 ºC. Flow Lac 100 (Lactose monohydrate, LIGAMED MF-2-V-MB (Magnesium Stearate) by Peter Greven as lubricant, and Colloidal Silicon dioxide (Aerosil 200) by

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Evonik as glidant were among the extra granular components. The tablet compressor was then used to compress the final blend material to prepare core tablets.

3.3.2 Preparation of the compression coat tablets

The composition comprising Methocel HPMC K100M DC2 (Hydroxy propyl methyl cellulose) by IIF as release retarding polymer and Methocel HPMC K15 M (Hydroxy propyl methyl cellulose) by IIF as release retarding polymer were used to prepare coat tablets by applying design expert software. Outer layer of core tablet was made up by direct compression method. For the preparation of coat tablets, the die of the tableting machine was filled manually with the half-weighed amounts of coating component. A core tablet was positioned in the center. The core pill was then covered with the remaining half of the powder. The type and concentration of polymer utilized to produce the coated tablets varied throughout formulations. Direct compression was used to create compressed core tablet system. Excipients were homogeneously blended and subsequently compressed into tablet using 12 station multi tooling rotary tablet punching machine.

Table 1: Formulation composition of Compression coated tablet

Sr. No.	Material	Quantity per Unit dose (mg)
Inner core tablet composition		
Intra granular materials		
1.	Salbutamol Sulphate	9.60
2.	Flow Lac 100 (Lactose monohydrate)	103.9
3.	Klucel EXF (Hydroxypropyl cellulose)	2.00
4.	Aerosil 200 (Colloidal Silicon dioxide)	1.00
5.	Milli Q Water	Q.S.
Extra granular materials		
6.	Flow Lac 100 (Lactose monohydrate)	30.00
7.	LIGAMED MF-2-V-MB (Magnesium Stearate)	2.00
8.	Aerosil 200 (Colloidal Silicon dioxide)	1.50
Total weight of core tablet		**150.00**
Mantle or Coating composition		
9.	Flow Lac 100 (Lactose monohydrate)	81.54
10.	Methocel HPMC K100M DC2 (Hydroxy propyl methyl cellulose)	107.64
11.	Methocel HPMC K15 M (Hydroxy propyl methyl cellulose)	53.82
12.	A-Tab (Dicalcium phosphate anhydrous)	82.00
13.	LIGAMED MF-2-V-MB (Magnesium Stearate)	1.50
14.	Aerosil 200 (Colloidal Silicon dioxide)	3.50
Total weight of compression coated tablet		**480.00 mg**

Based on the results obtained from preliminary studies the range of HPMC K100 M and HPMC K15 M were selected. The base design of experiment consisted of 15 runs to select the best optimised formulation.

4. Development of UV spectroscopic and RP-HPLC method for analysis of salbutamol sulphate:

The proposed UV spectroscopic method allows a rapid and accurate quantification of SAL from compressed coated tablets preparation with minimum time consuming for sample preparation. Moreover, the spectrophotometric method involves simple instrumentation compared with other instrumental techniques. The absorption spectra of SAL (9.6 ppm) in methanol are shown in Figure 1.

4.1. Selection of detection wavelength

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. In the present study, standard solution of Salbutamol sulphate was scanned in the range of 200 to 400 nm wavelengths. The drug showed maximum absorbance at 227 nm. Hence, the 227 nm wavelength was chosen for estimation of Salbutamol sulphate in bulk drug and final dosage form i.e. compressed coated tablets.

4.2. Selection of chromatographic condition

Appropriate selection of the RP-HPLC method depends on the nature of the sample which is ionic or ionizable or neutral molecule, its molecular weight and solubility. RP-HPLC was chosen for the initial separation because of its simplicity and suitability. To optimize the condition of chromatography, the effect of chromatographic variables such as: mobile phase, pH, flow rate, and solvent ratio were studied, and the chromatographic parameters such as capacity factor, asymmetric factor, resolution, and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

4.3. Selection of mobile phase ratio

The solution containing standard of salbutamol sulphate was chromatographed over mobile phases having different ratio of buffer and acetonitrile, at different pH values.

Mobile phase 10 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3.5 with orthophosphoric acid): Methanol: Acetonitrile in the ratio of 60: 30: 10% v/v/v was found to be optimum.

4.4. Selection of suitable column

For HPLC method, various columns are available commercially but the main aim was to separate the drug peak in the presence of degradation products peaks and other impurities. So, the Kromstar C_{18}

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column was selected over the other columns. Kromstar C_{18} column has embedded polar groups which are more stable at lower pH and high carbon loads, provides high peak purity and more retention to polar drugs and facilitates the separation of impurity peaks within a very short run time.

5. Method validation:
The methods were validated according to International Conference for Harmonization (ICH) guideline Q2 (R1 & R2) for validation of analytical procedures[10-11].

5.1 Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. It was proved by comparing the chromatogram of mobile phase, test preparation solution to show that there was no interference of mobile phase and excipients peaks. Based on these chromatographs, developed RP-HPLC proved its Specificity.

5.2 Linearity: The calibration curve was obtained with concentrations of the standard solutions (4.8 to 24 µg /ml) for both methods. The sets of solutions 4.8, 9.6, 14.4, 19.2, 24 µg /ml were prepared six times. The linearity was evaluated by regression analysis, which was calculated by the least square regression method.

5.3 Precision: Precision of these methods was checked by analyzing the samples 4.8, 9.6, 14.4 µg /ml at three different time intervals of the same day (intra-day precision) as well as on different days (inter-day precision). 9.6 ppm was run six times a day for repeatability.

5.4 Accuracy: The accuracy of proposed methods was determined by recovery studies. It was determined by recovery of known amounts of SAL reference standard 9.6 ppm added to the sample at the beginning of the process and all solutions were prepared in triplicate at 50%, 100% and 150% levels. For recovery studies proportion of SAL in in-house preparation, was carried out by adding reference standard SAL in to compressed coated tablet powder.

5.5 Limit of detection and limit of quantification: Limit of detection (LOD) and limit for quantification (LOQ) were calculated by using the values of slopes and intercepts of the calibration curves. Results showed in Table 2.

5.6 Assay: A buffer solution (pH 3.5 was prepared by dissolving 0.272 gm of potassium dihydrogen phosphate in 500 ml in Milli Q water; pH was adjusted to 3.5 with diluted orthophosphoric acid and filtered. The mixture of 10 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3.5 with orthophosphoric acid): Methanol: Acetonitrile in the ratio of 60: 30: 10% v/v/v were used as mobile phase and diluent, respectively. Standard stock solution of Salbutamol sulphate was prepared by accurately weighing 9.6 mg of salbutamol sulphate, which was transferred into a 100 ml volumetric flask, sonicated to dissolve and 1 ml diluted to make up the volume with 10 ml diluent and mixed well. The sample was assayed by using developed RP-HPLC method. Results of Assay showed in table 5.

5.7 Robustness: Robustness for RP-HPLC method was determined by analysis of samples under deliberately changed chromatographic conditions. The flow rate of the mobile phase was changed from 1ml/min to 0.8 ml/min and 1.2 ml/min while the ratio of the mobile phase was changed by ± 2%. The effect on retention time and peak parameter was studied. Results of Robustness showed in table 5.

6. RESULTS AND DISCUSSION:

6.1 UV spectroscopic method:

The proposed UV spectroscopic method allows a rapid and accurate quantification of SAL from compression coated tablets preparation with minimum time consuming for sample preparation. Moreover, the UV spectroscopic method involves simple instrumentation compared with other instrumental techniques. The absorption spectra of SAL in methanol are shown in Figure 1.

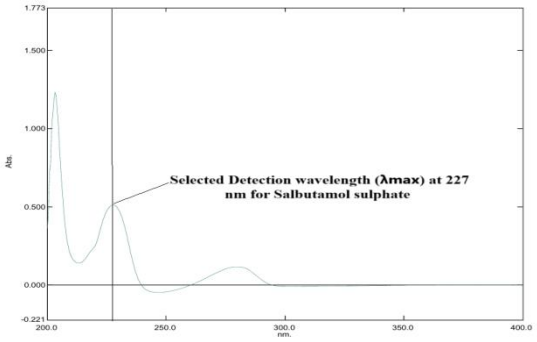

Figure 1: UV Spectra of Salbutamol sulphate (9.6 µg/ml) at 227 nm

Wavelengths selected for analysis are 227 nm (λmax of SAL). Calibration curves were constructed in the concentration range of (4.8 to 24 µg/ml) (Figure 2). Beer's law was found to be obeyed over this concentration range, and the coefficient of regression for SAL was found to be nearer to 1 (Table 2).

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Table 2: Linearity, LOD and LOQ data

Parameters	UV Spectrophotometric method	RP-HPLC method
Detection wavelength	227 nm	
Linearity & Range	4.8 to 24 µg/ml	4.8 to24 µg/ml
Regression equation	y = 0.0534x + 0.1765	y = 15775x + 50174
Regression Coefficient	0.9990	0.9996
Limit of detection (LOD)	0.41	0.30
Limit of quantification (LOQ)	1.23	0.90

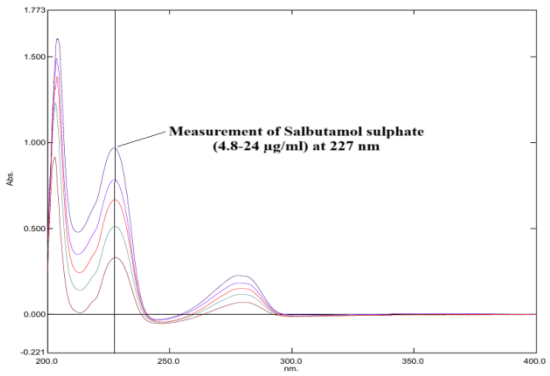

Figure 2: UV Spectra of Salbutamol sulphate (4.8 to 24 µg/ml) at 227 nm

Precision was calculated as inter-day and intra-day variations for SAL. Percent relative standard deviations for estimation of SAL under intra-day, inter-day and repeatability variations were found to be less than 2%. Table 3 shows the experimental values obtained for the Precision study.

Table 3: Precision data

Precision study of Salbutamol sulphate 227 nm				
Intra-day precision of Salbutamol sulphate				
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**	**Mean Area ±SD (n=3)**	**% RSD**
	UV Method		**HPLC Method**	
4.8	|-0.330| ± 0.0039	1.20	128615 ± 1509.97	1.17
9.6	|-0.493| ± 0.0040	0.82	197898.7 ± 1818.28	0.92
14.4	|-0.669| ± 0.0034	0.52	275723.7 ± 2052.51	0.74
Inter-day precision of Salbutamol sulphate				
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**	**Mean Area ±SD (n=3)**	**% RSD**
	UV Method		**HPLC Method**	
4.8	|-0.330| ± 0.0040	1.22	128348.3 ± 1527.53	1.19
9.6	|-0.493| ± 0.0043	0.87	198332 ± 2084.90	1.05
14.4	|-0.669| ± 0.0036	0.55	276057.7 ± 2003.36	0.73
Repeatability of Salbutamol sulphate				
Conc. (µg/ml)	**Mean Absorbance ±SD (n=6)**	**% RSD**	**Mean Area ±SD (n=3)**	**% RSD**
	UV Method		**HPLC Method**	
9.6	|-0.491| ± 0.0041	0.84	198545 ± 1973.20	0.99

A good & acceptable accuracy of the method was verified with a mean percent recovery in the range of 99.93% to 99.95% for UV Spectrophotometric method and 99.94% to 99.98% for PR-HPLC method. (Table 4).

Table 4: Accuracy data

Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Std Amt. (µg/ml)	Total amount Recovered (µg/ml)	% Mean Recovery ± SD (n=3)
UV spectroscopic Method	50	9.6	4.8	14.4	14.39	99.93±0.0056
	100	9.6	9.6	19.2	19.19	99.94±0.0057
	150	9.6	14.4	24.0	23.99	99.95±0.0058
RP-HPLC Method	50	9.6	4.8	14.4	14.392	99.94±0.0065
	100	9.6	9.6	19.2	19.192	99.95±0.0072
	150	9.6	14.4	24.0	23.996	99.98±0.0053

RP-HPLC method: Different proportions of acetonitrile, methanol and 10mM phosphate buffer (pH was adjusted to 3.5 using orthophosphoric acid) was tried for selection of mobile phase. Ultimately, 10mM phosphate buffer (pH adjusted to 3.5 with orthophosphoric acid): Methanol: Acetonitrile in the ratio of 60: 30: 10% v/v/v respectively was finalized as the optimum mobile phase. Figure 3 shows typical chromatogram obtained from the analysis of linearity of SAL using the RP-HPLC method.

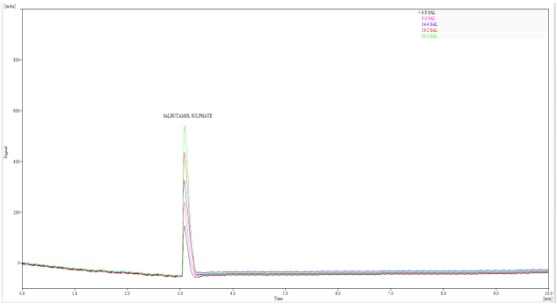

Figure 3: RP-HPLC chromatogram of Salbutamol sulphate (4.8 to 24 µg/ml) at 227 nm

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The elution order was SAL (Rt = 3.16 min), 6467 no. of theoretical plates, 1.02 tailing factor at a flow rate of 1.0 ml/min. The chromatogram was recorded at 227 nm as the overlay UV spectra of SAL showed maximum response at this wavelength.

The calibration curves for SAL were constructed by plotting concentration versus peak area and showed good linearity in the 4.8 to 24 µg/ml, and the coefficient of regression for SAL was found to be nearer to 1 (Table 2).

Precision was calculated as inter-day and intra-day variations for SAL. Percent relative standard deviations for estimation of SAL under intra-day and inter-day variations were found to be less than 2% (Table 3).

The accuracy of proposed methods was determined and the mean percent recovery was found in the range of 98%-102% which was found to comply as per ICH guideline Q2 (R1 & R2) (Table 4) indicating an agreement between the true value and found value. The experimental values obtained from assay and robustness for the determination of SAL in samples are showed in (Table 5). The %Assay in the range of 99.79% for UV and 99.89% for RP-HPLC method which was found to comply as per ICH guideline Q2 (R1 & R2). For robustness studies in all deliberately varied conditions, the RSD were found to be less than 2%.

Table 5: Assay and Robustness data

Assay and Robustness data			
Assay Data	**Amount in synthetic mixture (µg/ml)**	**Amount found (µg/ml)**	**% Assay ± S.D. (n=3)**
UV spectroscopic method	9.6	9.58	99.79±0.038
RP-HPLC method	9.6	9.59	99.89±0.013
Robustness Data			
Condition	**Variation**	**API**	**Dosage form**
		% Assay ± SD (n=3)	**% Assay ± SD (n=3)**
Flow rate (1 ml±0.2 ml/min)	0.8 ml/min	99.01±604.51	99.76±702.37
	1.0 ml/min	99.50±699.42	99.68±750.55
	1.2 ml/min	99.85±706.47	99.25±781.02
Detection wavelength (227 nm±2 nm)	225	99.76±680.12	99.29±709.45
	227	100.12±699.42	100.25±750.55
	229	99.75±702.04	99.92±763.76
Mobile Phase: Phosphate Buffer (pH 3.5): Methanol: ACN: (60:30:10 ±2 %v/v/v/v)	62:32:06	98.97±652.07	99.75±721.11
	60:30:10	99.75±699.42	99.86±750.5
	64:28:08	99.95±720.14	99.25±757.18

7. CONCLUSION:

The two proposed methods based on the UV spectrophotometry and RP-HPLC were developed and validated as per ICH guideline Q2 (R1 & R2). The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy for the proposed methods. Hence, it can be concluded that the developed spectrophotometric and chromatographic methods are accurate, precise, and selective and can be employed successfully for the estimation of SAL in bulk drug and compressed coated tablet dosage form.

The HPLC method developed and validated in this work proved to be simple, fast, accurate, precise, and sensitive. The method described affords quantification of Salbutamol sulphate. There are also advantages in terms of simplicity of sample preparation and the low costs of solvents used. So, the method described is specific. Hence, this method can be used for analysis of Salbutamol sulphate in bulk drug substance & pharmaceutical dosage form such as compression coated tablets.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

REFERENCES:

1. Prabakaran D, Singh P, Kanaujia P, Jaganathan KS, Rawat A, Vyas SP. Modified push–pull osmotic system for simultaneous delivery of theophylline and salbutamol: development and in-vitro characterization. Int J Pharm. 2004; 284:95–108. doi: 10.1016/j.ijpharm.2004.07.015.
2. Tripathi KD. Drugs for cough and bronchial asthma. In: *Essentials of Medical Pharmacology*. 6th ed. New Delhi: Jaypee Brothers Medical Publishers; 2008. p. 217–218.
3. Dave HN, Mashru RC, Thakkar AR. Simultaneous determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in pharmaceutical formulations using rapid derivative spectrophotometric methods. Anal Chim Acta. 2007; 597:113–120. https://doi.org/10.1016/j.aca.2007.06.035.
4. Mukherji G, Aggarwal N. Derivative UV spectroscopic determination of salbutamol sulphate in the presence of gelatin. Int J Pharm. 1991; 71:187–191. https://doi.org/10.1016/0378-5173(91)90389-6.

5. Mukherji G, Aggarwal N. Quantitative estimation of salbutamol sulphate by derivative UV spectroscopy in the presence of albumin. Int J Pharm. 1992;86(2–3):153–158. https://doi.org/10.1016/0378-5173(92)90192-5.
6. Mishra AK, Kumar M, Mishra A, Verma A, Chattopadhyay P. Validated UV spectroscopic method for estimation of salbutamol from tablet formulation. Arch Appl Sci Res. 2010; 2: 207–211. Available online at www.scholarsresearchlibrary.com.
7. Parimoo P, Umapathi P, Ilango K. Simultaneous quantitative determination of salbutamol sulphate and bromhexine hydrochloride in drug preparations by difference spectrophotometry. Int J Pharm. 1993; 100: 227–231. https://doi.org/10.1016/0378-5173(93)90095-W.
8. Kasawar GB, Farooqui M. Development and validation of stability-indicating RP-HPLC method for simultaneous determination of related substances of albuterol sulphate and ipratropium bromide in nasal solution. J Pharm Biomed Anal. 2010; 52:19–29. doi: 10.1016/j.jpba.2009.11.026.
9. Dave HN, Mashru RC, Patel AK. Thin layer chromatographic method for determination of a ternary mixture containing salbutamol sulphate, ambroxol hydrochloride and theophylline. Int J Pharm Sci. 2010; 2: 390–394.
10. International Conference on Harmonisation. *ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology*. Geneva: ICH; 2005.
11. International Conference on Harmonisation. *ICH Q2(R2): Validation of Analytical Procedures*. Geneva: ICH; 2023. Available from: https://www.ema.europa.eu.
12. Kalyani L, Rao CVN. Simultaneous spectrophotometric estimation of salbutamol, theophylline and ambroxol in three-component tablet formulation using simultaneous equation method. Karbala Int J Mod Sci. 2018;4(1):171–179. doi: 10.1016/j.kijoms.2018.01.004.
13. Gadekar G, Patil S, Shah R, Ghodke D. Development and validation of a simple UV spectrophotometric method for estimation of salbutamol sulphate from pharmaceutical formulations. Int J Curr Pharm Res. 2019;11(5):72–75. doi:10.22159/ijcpr.2019v11i5.35707.
14. Pandya HN, Berawala HH, Khatri DM, Mehta PJ. Spectrofluorimetric estimation of salbutamol sulphate in different dosage forms by inclusion complex with β-cyclodextrin. Pharm Methods. 2010;1(1):49–53. doi:10.4103/2229-4708.72231.
15. Rangari NT, More VS, Chumbhale DS, Kadam JN, Kalyankar TM, Muley YP. Development and validation of stability-indicating UV–visible spectrophotometric method for simultaneous determination of salbutamol sulphate and ambroxol hydrochloride in liquid dosage form. Int J Health Sci. 2022;6(S1):2744–2758. doi:10.53730/ijhs.v6nS1.5208.
16. Quintino MSM, Angnes L. Biamperometric quantification of salbutamol in pharmaceutical products. Talanta. 2004;62(2):231–236. doi:10.1016/j.talanta.2003.07.004.
17. Halabi A, Ferrayoli C, Palacio M, Dabbene V, Palacios S. Validation of a chiral HPLC assay for (R)-salbutamol sulphate. J Pharm Biomed Anal. 2004;34(1):45–51. doi:10.1016/j.jpba.2003.08.020.
18. Malkki L, Tammilehto S. Optimization of separation of salbutamol and its decomposition products by liquid chromatography with diode-array detection. J Pharm Biomed Anal. 1993;11(1):79–84. doi:10.1016/0731-7085(93)80151-P.
19. Bernal JL, del Nozal MJ, Velasco H, Toribio L. HPLC versus SFC for determination of salbutamol sulphate and its impurities in pharmaceuticals. J Liq Chromatogr Relat Technol. 1996;19(10):1579–1589. doi:10.1080/10826079608005493.
20. Shaikh NK, J. R, Bhangale JO. Analysis of Vildagliptin and Nateglinide for simultaneous estimation using spectro-chromatographic methods. Eur J Mol Clin Med. 2020;7(8):741–755.
21. Shaikh NK, Jat R, Bhangale JO. Development and validation of stability-indicating RP-HPLC and UV method for simultaneous quantitation of repaglinide and sitagliptin phosphate. Am J PharmTech Res. 2020;10(6):95–114. doi:10.46624/ajptr.2020.v10.i6.007.
22. Momin MAM, Hossain MF, Begum AA, Roy J, Anisuzzaman SM. Development and validation of stability-indicating assay method of salbutamol sulphate metered dose inhaler by HPLC. Int J Pharm Phytopharmacol Res. 2013;2(6):439–448.
23. Fareed S, Sethi V, Siddiqui A, Tyagi L. HPLC method development and validation for estimation of salbutamol sulphate. J Adv Sci Res. 2019;10(3):57–62.
24. Harps LC, Jendretzki AL, Wolf CA, Girreser U, Wolber G, Parr MK. Development of an HPLC-MS/MS method for chiral separation and quantitation of (R)- and (S)-salbutamol and their sulfoconjugated metabolites in urine. Molecules. 2023;28(20):7206. doi:10.3390/molecules28207206.
25. Muralidharan S, Kumar J. High performance liquid chromatographic method development and validation for salbutamol. J Pharm Res Int. 2012;2(4):228–237. doi:10.9734/BJPR/2012/1279.
26. Duong D, Nguyen NTY, Nguyen HQ, Nguyen DC, Uyen TDT, Ca TT. RP-HPLC method for determination of salbutamol and bromhexine in syrup: optimization by response surface methodology. Asian J Chem. 2020; 32:3135–3143.
27. Sule S, Bhure SP, Sule A, Ambadekar SR. A simple and rapid HPLC method for assay of salbutamol, ciprofloxacin and mannitol in triple-combination dry powder formulation. Am J Chem. 2023;13(4):97–101. doi:10.5923/j.chemistry.20231304.01.
28. Tyagi A, Sharma N, Mittal K, Mashru R, Bhardwaj T, Malik J, et al. HPTLC-densitometric and RP-HPLC method development and validation for determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in tablets. Pharm Anal Acta. 2015;6(3):1–10.
29. Elkady Y, El-Adl SM, Baraka M, Sebaiy MM. Analytical methods for determination of salbutamol, ambroxol and fexofenadine. J Biotechnol Bioprocess. 2020;1(1). doi:10.31579/2766-2314/004.
30. Kumar Y, MK, SH M. Formulation and evaluation of salbutamol sulphate taste-masked oral disintegrating tablets. Int J Pharm Sci Nanotechnol. 2021;14(4):5571–5576.