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Analytical QbD based systematic development of a novel RP-UHPLC method for the quantification of albuterol sulphate in its metered dose inhaler formulations

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ABSTRACT: Albuterol sulphate is a Beta-2–adrenergic agonist drug mainly used in the form of nasal inhalations to treat chronic bronchitis and chronic obstructive pulmonary disease (COPD). A simple, rapid, precise chemometrics-based RP-UHPLC method has been developed and validated in its MDI formulations by the QbD approach. The chromatographic separation was achieved by Hibar HR purospher STAR RP-18 encapped column (C18, 250 mm × 4.6 mm, 5 µm) with UV detection λ_{max} 225 nm. The retention time (Rt) for albuterol sulphate was observed as 2.434 over a run time of 5 minutes. Optimization of the method for the quantification of albuterol sulphate in its metered dose inhaler formulations has been performed by using 2^2 central composite design with response surface methodology that might lessen the solvent consumption, duration, and additional resources. A total of two independent variables, organic phase composition, and flow rate, were analyzed to construct a quadratic process model to examine the effect of independent variables or critical material attributes (CMAs) ahead the observed responses known as the critical method parameters (CMPs) within the designed space. The optimized UHPLC method contains phosphate buffer (10 mM KH_2PO_4) and acetonitrile (60:40 %v/v) as the mobile phase, which was delivered with a flow rate of 1.0 ml/min and the column was maintained at 40°C. The developed QbD based method was subsequently validated as per ICH Q2 R1 guidelines. The method obeyed linearity within the range of (5-120 µg/mL) with regression R^2 of 0.999 for albuterol sulphate. The low coefficients of variation obtained in the intraday and inter-day precision studies indicate précised method. High recovery of albuterol sulphate has been observed within the range of (98.0-102.0 %). The developed method can be intensely employed as a highly robust, accurate, and cost-effective for routine analysis in research labs that competitively separate the drug from its excipients.

KEYWORDS: Accuracy; validation; isocratic; experimental design; albuterol sulphate; correlation coefficient.

1. INTRODUCTION

Albuterol sulphate is chemically known as 4-[2-(tert-butyl amino)-1-hydroxyethyl]-2 hydroxymethyl) phenol sulfuric acid represents the class of drugs known as short-acting beta-2–adrenergic agonists. Albuterol sulphate (Figure 1) is actively being used in the management of bronchospasm in bronchial asthma, chronic bronchitis, and emphysema, prophylaxis of exercise-induced asthma as well as COPD. Albuterol sulphate relaxes all airway smooth muscles, from the trachea to the incurable bronchioles, consequently making breathing easier [1]. As per the report of the World Health Organization (WHO), the name for albuterol base is known as salbutamol. It is a white to off-white crystalline solid which is soluble in water and faintly soluble

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in ethanol. It is employed for a range of bronchial disorders, in rhinitis, and as an anti-arrhythmic. It blocks the muscarinic cholinergic receptors without specificity for subtypes, resulting in a decrease in the formation of CGMP [2]. It is generously soluble in methanol and water, but at a little bit sparingly soluble in ethanol, but insoluble in lipophilic solvents such as ether, chloroform, and fluorocarbons. The formulations containing albuterol sulphate are used in the management of chronic obstructive pulmonary disease (COPD) and asthmatic disorders. The approaches of Quality by Design (QbD) principles in Research laboratories can help to develop an analytical method through a systematic approach, providing a significant advance over the traditional heuristic and empirical methodology [2,3]. The method was divided into operative units, and for each unit, critical method variables affecting the results were identified. A risk analysis was performed to select critical method parameters (CMPs) that should be introduced in the design of experiments (DoEs) [4].

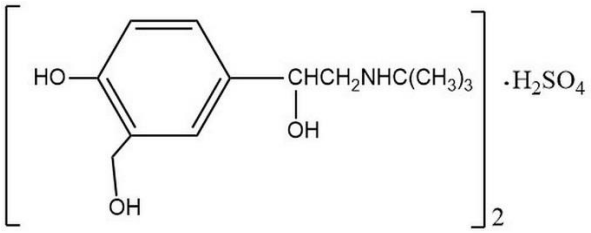

Figure 1. Chemical structure of albuterol sulphate.

Chemometrics has been increasingly viewed as extensive beyond price aspects of this technique since an enormous number of variables might be concurrently controlled to achieve the preferred separations. Moreover, their applicability might precisely predict, recognize, and optimize the momentous factors to carry out the competent outcomes through partial experimental trials [4-6].

An extensive literature survey revealed few HPLC [3-6] methods with combination dosages had been reported. But notably, no specific method has been reported with ATP, risk assessment, and method operable design region (MODR) concepts, according to FDA concern, regulatory flexibility, using Analytical QbD (AQbD) paradigm. Apart from this, the Ultra High Performance Liquid Chromatography (RP-UHPLC) can be pretty advantageous than other conventional HPLC techniques for its utmost speed, sensitivity and resolutions to obtain quality-based first-rated analytical results [7-9]. Therefore, a scientifically determined effort was implemented to produce a novel QbD based RP-UHPLC systematic development for the quantitation of albuterol sulphate in its marketed formulations of MDI with superior robustness.

2. RESULTS AND DISCUSSION

2.1. AQbD based statistical design and interpretation

The optimization for the data analysis was in point of fact take on by multivariate linear regression analysis with the aid of Design-Expert® (Version 13), Stat-Ease Inc., Minneapolis, MN, advanced statistical software of USA, for screen out the experimental responses with the second-order quadratic polynomial model for method optimization and the evaluation of its parameters along, significant impact and interaction effects and randomized the runs [10, 11]. The statistical linear regression analysis was done by Microsoft excel 2019 Software (Microsoft, USA). By the principles of the chemometrics approach, to precisely optimize the substantial prominent factors in their combination, these series of suitable experimental designs have to be built-in as well as to interpret the experimental procedures [12]. At first, repeated, varied practice attempts were executed to expand the understanding of the method performance and recognition of various vital independent factors and their impact on the dependent variables. The Six-Sigma approach put into a systematic practice for quality performance indicator in separating the components through chromatography has summarized in diverse literature aspects [12-14].

2.2. Method development by central composite design (CCD)

The central composite design was incorporated to estimate the effects of independent chromatographic parameters upon the two defined critical analytical attributes (CAAs), retention time, peak area as well as the USP plate count. The design comprehends a total of 13 experimental runs. The trial runs aids in configure of statistical data's through response surface methodology with intent analysis of critical factors by assessing their leading effect to obtain the crtical method parameters (CMPs) [13-16]. The design matrix of all the

encoded critical quality factors and its associated reponses are summarized in Table 1. Similarly, Table 2 denotes regarding particulars of independent variables i.e., organic phase composition and flow rate with their deliberate limits and its observed responses (CAAs).

2.2.1. Optimized Chromatographic Conditions

Optimized Trail: Concentration (20 µg/mL)
Column: Hibar HR purospher STAR RP-18 (C18, 250 mm × 4.6 mm, 5 µm)
with UV Detection at 225 nm
Injection volume: 20 µl
Sample temperature: 5°C
Column temperature: 40°C
Flow rate: 1.0 ml/minute

Mobile phase: The required mobile phase composition was found to be phosphate Buffer [10mM KH_2PO_4] (600ml): acetonitrile (400ml) with a ratio of (60:40% v/v). The optimized chromatographic conditions and the optimized trails of the standard and samples (20µg/mL) for the developed methods are demonstrated in Table 3 and Fig. 2 (a-d), respectively.

Table 1. Experimental design matrix for factors and their obtained responses by 2^2 central composite design.

Factors	Name	Units	Type	Minimum	Maximum	Coded low	Coded High	Mean	Std. dev
A	Organic Phase Composition	(% v/v)	Numeric	10	70.00	1−40.00	1−70.00	40	0.7071
B	Flow rate	(ml/minute)	Numeric	0.5	1.5	1−0.5	1−1.5	1	0.7071

Table 2. Experimental runs of selecting (2^2) factors by central composite design.

	Factor 1	**Factor 2**	**Response 1**	**Response 2**	**Response 3**
Run	A: Organic phase (%v/v)	B: Flow rate (ml/minute)	Peak area (cm^2)	Retention time (minute)	USP plate count
1	0	0	1704810	4.98	1500
2	0	0	1558935	2.15	5466
3	0	0	1134906	3.68	4254
4	1	1	66201	1.27	8200
5	0	-1	1890678	4.97	2869
6	0	0	1956743	3.74	2200
7	-1	1	1024612	1.86	1205
8	0	0	1408659	3.06	4587
9	-1	-1	1697864	3.24	2456
10	-1	0	1856452	4.65	3976
11	0	1	285595	2.434	3116
12	1	0	135051	1.92	4503
13	1	-1	1503879	3.82	3689

2.3. Optimization of chromatographic method using response surface methodology (RSM)

After selecting optimum chromatographic conditions, the CCD with response surface methodology (RSM) was executed by applying the ANOVA principles for ascertaining an optimized experimental routine environment for the developed analytical method [15,16]. From the applications of central composite design, two vital critical method parameters (CMPs) were found out as a consequence and being analyzed based on their significant effect on the chosen responses or desired dependent variables (CAAs). Organic phase composition (X1) and flow rate (X2) were selected most influential factors for optimization. A sum of 13 experimental runs were subjected as per the Design Matrix emphasized in Tables 1 and 2. A robust, accurate,

AQbD based RP-UHPLC method was developed to quantify the albuterol sulphate in its aerosolized medication. A mixture of phosphate buffer (10mM Potassium dihydrogen phosphate) and acetonitrile (60:40 %v/v) was employed as the mobile phase with an optimal flow rate of 1 ml/minute.

The results of p-value, f value, and lack of fit statistics with post prediction, confirmation data's through principles of ANOVA by the implementation of CCD have been demonstrated in Table 4. The obtained equations of all the dependent variables or CAAs are enlisted below.

2.4. Interpretation of Response Surface Analysis (2-D and 3-D plots)

2.4.1. Effect of the factors organic phase and flow rate on peak area

Figure 2a & b depicts that, when the low coded level [0 to - 1] of flow rate (0.5ml/min) and the organic phase [-1 to 1] (10 % V/V) were taken, then it indicates the prevalence of light red region from 500000 to 1956743 cm^2. When the level increases from 0 to 1; both in the mobile phase and flow rate, it is shown by a light Green color region with a peak area of 20,00000 cm^2 (2E+06), however for the optimized response which was obtained, is close to 285595 cm^2, which indicates the green color region. The predicted R^2 and the adjusted R^2 were found to be 0.4157 and 0.6012; the difference among them less than 0.2, which can be suitably used to navigate the design space.

Table 3. Optimum chromatographic conditions and Linearity data's of albuterol sulphate.

Optimum Chromatographic Conditions		**Linearity Data**		
	Run time		**5 minutes**	
Retention time	2.434 minute	**Sl. No.**	**Predicted Conc. (µg/mL)**	**Peak area**
Flow rate	1 ml/minute	1	5	66190
Linearity range	(5 –120) µg/mL (R^2=0.999)	2	10	132058
Accuracy	% Recovery: Within [98-102%] Relative Standard Deviation (RSD < 2%)	3	20	285595
Precision	Relative Standard Deviation (RSD < 2%)	4	40	567453
LOD	1.38 µg/mL	5	80	1134906
LOQ	4.20 µg/mL	6	100	1427976

LOD: Limit of detection; LOQ: Limit of quantification

Table 4. ANOVA and its significance value with respect to quadratic model with post prediction and confirmation data of experimental design.

Source	**Peak area (cm^2)**		**Retention time (minute)**		**USP plate count**			
	F value	**P- value**	**F value**	**P- value**	**F value**		**P- value**	
Model	10.04	0.0041*	4.13	0.0493*	3.86		0.0499*	
A-Mobile phase	7.52	0.0208*	1.26	0.2885*	6.40		0.0322*	
B-Flow rate	12.57	0.0053	7.00	0.0245	1.03		0.3372	
AB	0.1638	0.6978	0.1176	0.7418	4.16		0.0718	
Lack of fit	2.53	< 0.0001*	0.8793	0.5780*	0.4762		0.7817*	
Run 11 Response	**Predicted Mean**	**Predicted Median**	**Observed values**	**Std Dev**	**SE Mean**	**95% PI low**	**95% PI high**	
Peak Area	628694	1755221	285595	427890	211184	-434500	1691888	
Retention Time	2.1357	8.61503	2.434	0.997866	0.492505	-0.343769	4.6152	
USP plate count	4278.42	4278.42	3116	1412.34	697.056	715.558	7841.29	

*Significant levels, i.e., less than α value (0.05); *P. I: prediction interval, Std Dev: standard deviation, SE: standard error

From the above ANOVA table the calculated equations for the observed responses are calculated as following equations 1, 2, 3:

Peak Area (Y1)= +1.248E+06-4.790E+05 A-6.193E+05 B (Eq.1)

Retention time (Y2)= 3.21338 + -0.456667 x A + -1.07767 x B (Eq.2)

USP plate count (Y3)=3693.92 + 1459.17 * A + 584.5 x B + 1440.5 x AB (Eq.3)

2.4.2. Effect of factors organic phase and flow rate on retention time

Figure 2c & d depicts that, when the range of low level [0 to -1] of the organic phase (10 %V/V) and flow rate (0.5ml/minute) was taken, then it indicates the prevalence of light yellow to the green colour region appeared from 4 to 4.8 minutes. By gradually increasing the levels from 0 to 1, there is quite an increased in retention time (Rt) up to 5 minutes, which shows the dark green region. However, the level between [-0.5 to 0] of flow rate and the mobile phase indicates the light blue area for optimized response, i.e., 6.790 minutes. This model is perfectly applicable to navigate the design space.

2.4.3. Effect of factors organic phase and flow rate on USP plate count

Figure 2e & f depicts that, when there is a high level [0 to +1] of mobile phase (70%V/V) and high level of flow rate (1.5 ml/minute) was employed; then it designates the occurrence of light blue to dark blue color region from 3000 to 2000 signifying a substantial decline in USP plate count. But gradually decrease of level parameter, i.e., from [0 to -1] then, it shows, the rise of plate count, which ranges from 4000 to 6000, indicates the yellowish red region. Hence the optimized response was found to be 3366, which is the in-between level of [0 to -0.5].

2.4.4. Counter (2-D) plot predicted and acutal values analysis of responses

The 2-D statistical counter plot depicts that predicted values for the obtained responses (Retention Time, Peak Area, and USP Plate count) are almost closer to the actual desired values indicating better accuracy and precision values for the observed responses (CAAs), which is passing throughout the central axis (R^2). The counter-plot analysis of the Predicted vs. Actual values of observed variables or desired responses are depicted in Figure 3 (a-c), respectively.

3. Analytical method validation

Analytical Method validation (AMV) is a procedure of performing numerous assessments designed to verify that an analytical test system is suitable for its intended reason and is capable of providing useful and legitimate analytical data or validation data that are ultimately playing an elementary role in the pharmaceutical industry [17,18]. Effect of input variables on the chromatogram characteristics was calculated for the selection of Critical Method Parameters (CMPs) and the ICH guidelines ICH Q2(R1). The developed analytical method by analytical QbD (AQbD) pattern has consequently been validated in terms of linearity, precision, accuracy, system suitability testing, robustness, and the limit of detection, limit of quantitation as per ICHQ2R1 recommended guidelines.

3.1. Linearity

The linearity identification of an analytical method is its potential to bring out the consequences which might be at once, or with the help of fine described mathematical adjustments, proportional to the analyte concentration within a specified range. Standard solutions of albuterol sulphate in the working range of 5–120 ppm were prepared in 10ml volumetric flasks by taking suitable aliquots from the stock solution and diluted up to the mark diluents 20 (µl) microliters of dilution were injected into the UHPLC column, and the drugs in the eluents were measured λ_{max} at 225 nm. The mean peak area was distinguished from the obtained chromatograms, and a curve of concentration vs. chromatographic peak area was constructed. Further, the linearity plot's regression equation was computed by the least-squares method [19, 20].

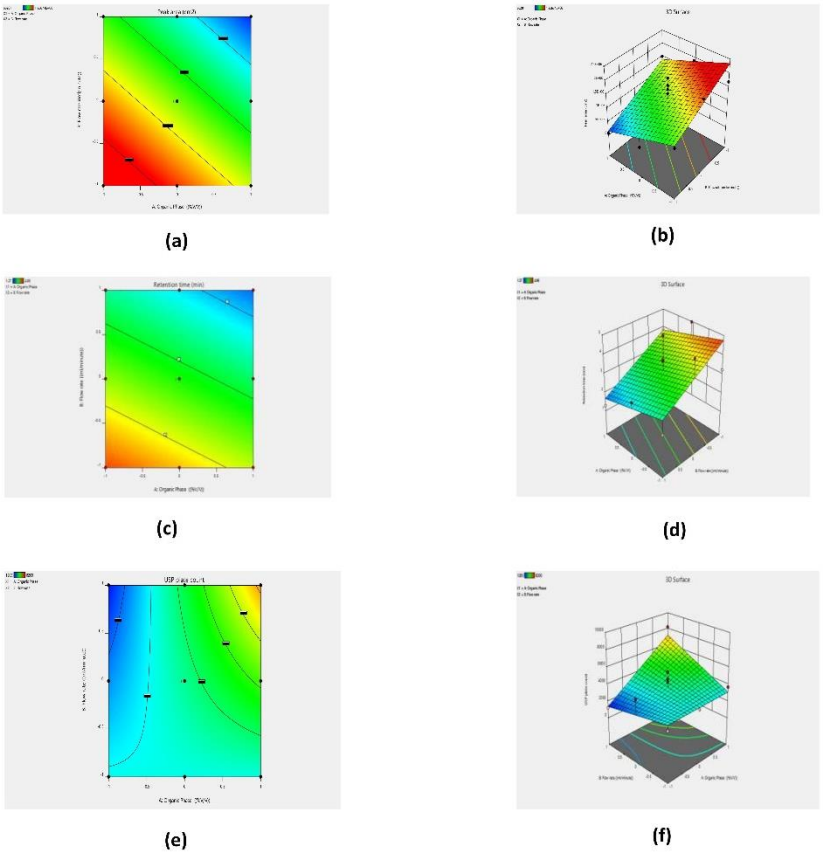

Figure 2. 2-D surface contour plot analysis of peak area response (a), 3-D surface contour plot analysis of peak area response (b); 2-D surface contour plot analysis of retention time response (c), 3-D surface contour plot analysis of retention time response (d); 2-D surface contour plot analysis of plate count response (e), 3-D surface contour plot analysis of plate count response (f).

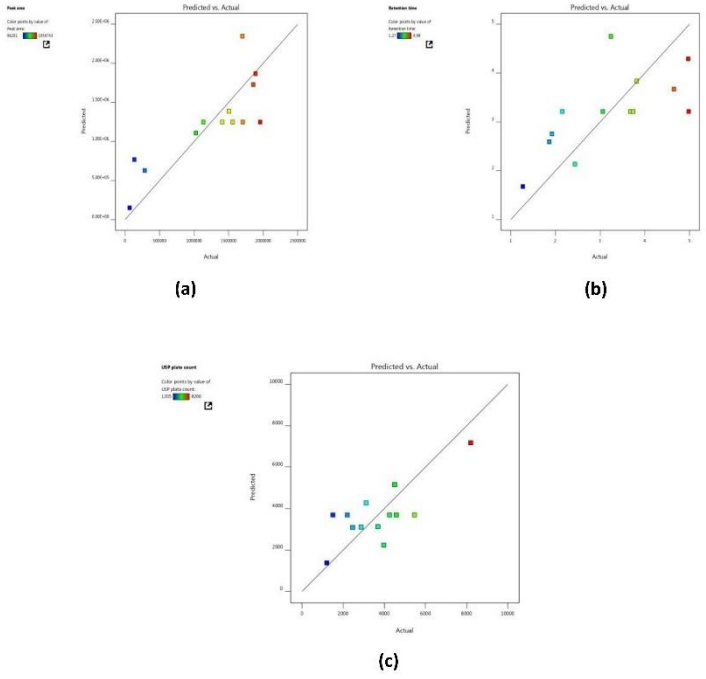

Figure 3. The counter plot analysis of the Predicted vs. Actual values of Peak area (a), Retention time (b), Plate count (c).

3.2. Precision

The precision parameter was studied in terms of repeatability (intra-day) and intermediate precision (inter-day) by injecting six standard preparations of albuterol sulphate; each injected six times on the same day (intra-day assay) and on a different day (interday assay) [21]. The % RSD was calculated for assay in repeatability and intermediate precision study accordingly.

3.3. Accuracy

The accuracy or recovery studies of the process were estimated by duly and appropriate diluting the sample (inhalation) solution to acquire concentrations analogous to 50%, 100%, and 150% levels of the drug albuterol sulphate [22,23]. Three standard preparations were prearranged at each level and analyzed. Ultimately the % recovery was pre-meditated and obtained from the amount recovered by comparing the average peak areas obtained for standard and solutions [24].

3.4. Robustness

A study was focused on perceiving the upshot of deliberate variations or by bringing minor variations in parameters, especially the [25, 26] optimized flow rate and column oven temperature in the optimized chromatographic conditions like flow rate (0.8 & 1.2 ml/min) and column temperature 40$\pm$ 5 °C (35°C and 45°C). Albuterol sulphate standard and sample solutions were evaluated at the altered conditions and the effect [25,26].

3.5. System suitability

The System suitability test (SST) is performed in order to recognize that the process is appropriate for the obligatory objectives and conditions the day the analysis was carried out. It is an intense confirmation factor to authenticate the quality of the process for accurate dimensions by spiking the six replicates of standard injections [26]. The SST was intended for diverse parameters such as theoretical plates, resolution, tailing factor, LOD, and LOQ, etc. [27].

3.6. Specificity

The specificity of the developed analytical method can be noted down in the presence of excipients present in the formulations. Specificity is the ability to determine the analyte unequivocally in the presence of components that may be expected to be present [28]. For interference from the excipients in nasal inhalation, a placebo mixture of the commonly used ingredients in inhalations was prepared and injected into the UHPLC system.

3.7. LOD & LOQ

The limit of detection (LOD) and limit of quantification (LOQ) of the current investigation were evaluated from the baseline noise of albuterol sulphate through comparisons of measured signals of samples with known analyte concentrations with that of the blank by (signal-to-noise) S/N ratio 3:1 (LOD) & 10:1 (LOQ) as per direction and procedure recommended by ICHQ2B guidelines [27-29].

3.8. Results of method validation parameters

3.8.1.Linearity

The linearity study was carried out by injecting a series of standards (SIX) of diluted stock solution of albuterol sulphate using the Solvent, at a minimum of five diverse concentrations in the range of 5–120 %, and the calculated regression equation was found to be Y= 14243x–4165 regression equation R^2 =0.999 which indicates the concentration was linear. The calibration curve data of the drug albuterol sulphate are represented in Table 3 and Figure 4, respectively.

3.8.2.Precision

The precision determines a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. The %RSD values were calculated for assay in repeatability, and the intermediate precision study was within the acceptance limit, i.e., Not more than 2%. The detailed data of precisions (system, intra-day, and inter-day) studies are demonstrated in Table 5.

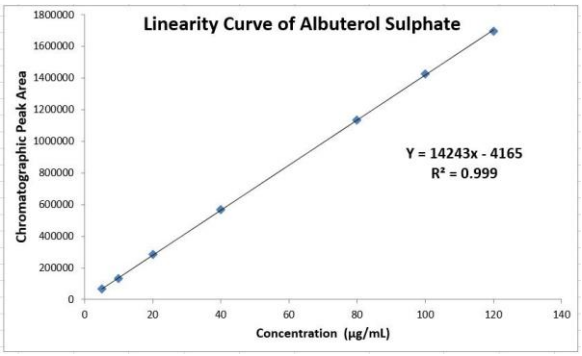

Figure 4. Calibration curve of Albuterol Suphate.

3.8.3. Accuracy

Accuracy is calculated by spiking the sample matrix of curiosity with a known concentration of analyte standard and same time analyzing the sample by means of method being validated. The recovery has performed at three levels, and the % recovery was observed within 98-102%. The obtained data of recovery studies are demonstrated in Table 6.

Table 5. System, Intra-day and inter-day precision data's of albuterol sulphate.

System Precision					Intra-day Peak Area at different time intervals (Day 1)			Inter-day Peak Area at different time intervals (Day 2)	
Conc. (µg/ mL)	Peak Area	Average	SD	%RSD	Conc. (µg/mL)	10 A.M	2 P.M	Conc. (µg/mL)	10 A.M	2 P.M
20	2624281	2638582	18294.46	0.6933	20	2734281	2745769	20	2738291	2749187
20	2623222				20	2753457	2766243	20	2757225	2754311
20	2619265				20	2693686	2689258	20	2699269	2710213
20	2656714				20	2691724	2695576	20	2698724	2756786
20	2648747				20	2714237	2736249	20	2718747	2788369
20	2659264				20	2729442	2718737	20	2729269	2719276
					Average	2719471	2725305	Average	2722919	2722919
					SD	24232.05	29802.02	SD	22398.41	28230.53
					%RSD	0.89	1.09	%RSD	0.82	

Table 6. Accuracy data of albuterol sulphate.

Accuracy data level	Amount of drug added	Amount of drug found	% Recovery
S1: 50 %	10	9.94	99.40
S2: 50 %	10	9.96	99.60
S3: 50 %	10	9.98	99.80
S4: 100 %	20	19.86	99.30
S5: 100 %	20	19.79	98.95
S6: 100 %	20	20.14	100.70
S7 :150 %	30	29.96	99.86
S8: 150 %	30	29.89	99.63
S9: 150 %	30	30.08	100.26
		Mean =	**99.72**
		SD =	**0.521**
		% RSD =	**0.52**

*Std Dev: standard deviation, RSD: Relative standard deviation

3.8.4. Robustness

Albuterol sulphate standard and sample solutions were evaluated at the altered conditions or deliberate variations of the drug sample to be analyzed. The significant effects of change in flow rate, the composition of the organic phase, and the variations in wavelengths are demonstrated in Table 7.

Table 7. System suitability test and Robustness data of albuterol sulphate.

System Suitability Test (SST)				Robustness Study						
Peak Area Injections	Optimized Condition (SST)	USP Plate Count	Tailing Factor	Parameter	Flow Rate [1+ 0.2 ml/minute]	Flow Rate [1-0.2 ml/minute]	Wavelength [225 +2 Nm]	Wavelength [225–2 Nm]	Amount of [ACN + 2%V/V]	Amount of [ACN -2%V/V]
Peak Area (Inj.1)	284391	3324	1.31	Peak Area (Inj.1)	274365	285595	272465	268476	285267	276253
Peak Area (Inj.2)	285198	3247	1,29	Peak Area (Inj.2)	275947	283997	274381	267429	280178	271122
Peak Area (Inj.3)	283816	3371	1.26	Peak Area (Inj.3)	272863	281359	274972	264419	282659	274337
Peak Area (Inj.4)	284675	3298	1.37	Mean	274391	283650	273939	266774	282702	273904
Peak Area (Inj.5)	289415	3310	1.32	Std	1542.17	2139.17	1310.55	2106.16	2544.76	2592.76
Peak Area (Inj.6)	287397	3281	1.29	% RSD	0.56	0.754	0.47	0.78	0.94	0.94
Mean	285815			Difference with respect to SST (%)	**4.0**	**0.8**	**4.2**	**6.7**	**1.1**	**4.2**
Std % RSD	2152.52 0.753									

*Std Dev: standard deviation, SST: System suitability test
RSD: Relative standard deviation, Inj: Injection

3.8.5. System suitability

System Suitability Test (SST) is primarily executed during the research to ensure that, the absolute analytical method is acceptable for deliberate applications. In this study, the SST was applicable in diverse parameters such as theoretical plates, resolution, tailing factor, etc. The chromatographic datas of SST for six standard injections are demonstrated in Table 7.

3.8.6. Specificity

Specificity study was performed by taking the standard drug solutions of albuterol sulphate MDI, which were injected separately to find any starting materials or excipients. From the results of placebo solutions, it has been observed that there is no interference of excipients at the retention time (Rt) of albuterol sulphate. The resulting chromatograms at 225nm showed clear separation, represented in 2d of Figure 5 (a-d) respectively [28,29].

3.8.7. LOD and LOQ

LOD and LOQ values are estimated manually by integrating the Signal to Noise (S/N) ratio of the lowest known concentration of linearity samples, and it will be articulated in µg/mL. The obtained values of the LOD was found to be 1.38 µg/mL, and LOQ was obtained as 4.20 µg/mL, respectively, which were demonstrated in Table 3.

3.9. Assay of pharmaceutical formulations (MDI)

Formulations containing albuterol sulphate, Metered-dose inhaler (MDI) valves were fitted or geared up by unified dischargement of two leveled dosages to waste into the air. Later on, the aerosol canister was allowed for persistent washing with organic solvents like methanol and subjected to drive for 2 to 3 minutes through compressed air. In a tiny vessel, a base containing stainless steel Plate, which bears three legs, with a central circular indentation having a whole of dimension 1.5 mm in diameter, was located and handled with care. At the vessel, within a time limit of about 2-3 minutes, the compacted or pressurized container was allowed for shaking, time to time. A total of 10 deliveries were ejected beneath the solvent surface, actuating the valve at intervals minimum of 5 to 10 seconds, preserving the pressurized vessel in a vertical plane, and releasing the pressurized-inhalation with the help of a whole in the central point of the base plate. Later on, the pressurized container was taken out, subsequently washed, and rinsed with diluents. The resulting collective solution, along with washings, was next undergone dilution to 50 ml. The representative data's of assay of two pharmaceutical formulations containing albuterol sulphate MDI has been reported in Table 8.

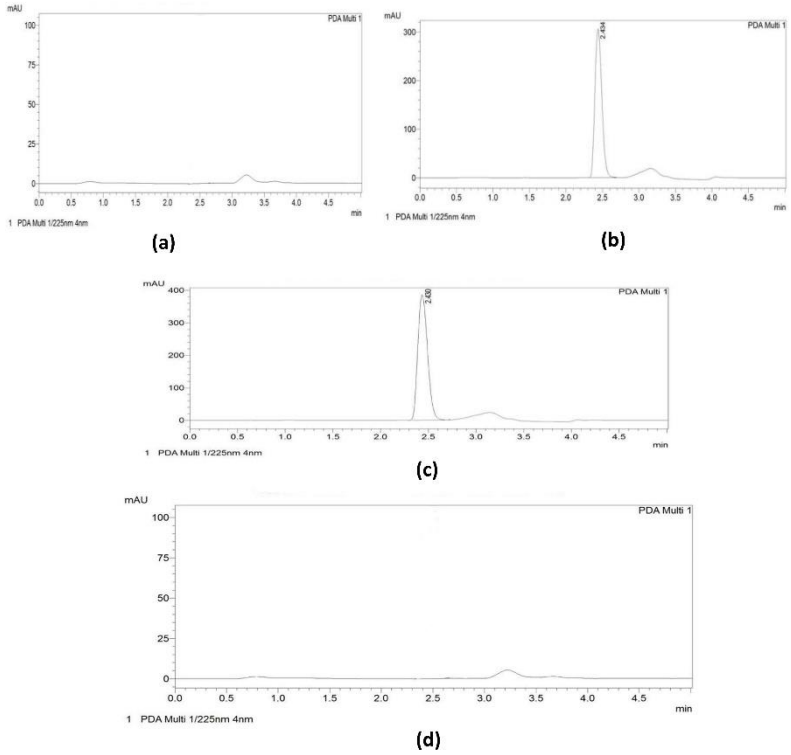

Figure 5. The optimized trails of the blank (a), standard (b), samples (c) and placebo (d) (20µg/mL).

Table 8: Assay of Marketed MDI Formulations.

Assay of Formulations			
Brand Names	**Label Claim (mcg)**	**drug obtained**	**% Recovery**
Brand I (Ventolin HFA)	90	90.16	100.17
Brand II (Pro Air HFA)	90	89.88	98.97

4. CONCLUSION

An economical, reliable analytical QbD (AQbD) based RP-UHPLC method has been developed that is found to be specific, sensitive, precise, and robust for the quantification of albuterol sulphate in bulk drugs and its MDI formulations without any interference from excipients. The chemometrics assisted chromatographic method ensures robust and efficient methodology, saving time, reagents, and extra resources. The proposed method could also be successfully applied for the routine quality control analysis of albuterol sulphate in its pharmaceutical dosage forms.

5. MATERIALS AND METHODS

5.1. Chemicals and reagents

Reference standard samples of albuterol sulphate (purity 99.4 % w/w) were obtained from Sun Pharmaceutical Laboratories Pvt Ltd. (Gujarat). The commercial formulations were procured from the local market. Acetonitrile, methanol, and OPA (HPLC grade), TBHS Buffer (AR grade) were purchased from Merck Laboratories Pvt. Ltd., Mumbai. HPLC grade water prepared from the Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the study. The Potassium dihydrogen phosphate (KH_2PO_4) was obtained from Fischer Scientific, Mumbai, India.

5.2. Instrumentation

An Agilent RP-UHPLC (advanced version of HPLC) with 1290 infinity II, HDR was used for analysis. The instrument was equipped with a quaternary gradient pump, PDA detector, an autosampler, and a column heating oven were used for the investigation. A Hibar HR purospher STAR RP-18 encapped column (C18, 250 mm × 4.6 mm, 5 µm) with UV Detection at 225 nm was employed for the development of analytical method. The chromatographic analysis and data acquisition were monitored using empower software. The mobile phase was used for degassing by selective assistance of Remi Instruments, Mumbai, India. PCI bath ultra-sonicator, a Sartorius SPA 225D electronic balances were used for weighing the materials. Filtration was carried out by the Nylon filter (0.45 µm)–Millipore, Mumbai, India. The pH measurements were made using a Metsar pH meter.

5.3. Methods

5.3.1. Preparation of standard stock solution:

10 mg of pure drug albuterol sulphate was weighed specifically and shifted into a 100ml volumetric flask. Methanol was added as a diluent with a distinct quantity (20 ml) to solubilize the drug. Later on, the above flask was appropriately shaken and allowed for sonication for 15 minutes. The requisite volume was then adjusted to the mark by means of diluent (methanol) in sequence to get the concentration up to 100 µg/mL.

5.3.2. Preparation of buffer

10mM TBHS (Tetra butyl Hydrogen Sulphate) Buffer in water (1000ml) was prepared as per official monographs. The pH was adjusted to pH 3 using Ortho Phosphoric acid (OPA).

5.3.3. Preparation of Mobile Phase

Required quantity of solvents like 600 ml buffer and 400 ml acetonitrile (ACN) were mixed adequately and ultasonicated for 5 minutes as mobile phase.

5.3.4. Final standard preparation

From the stock solution (100 mcg/ml), 2 ml was transferred to a 20 ml flask, with adjusted volume up to the required mark (mobile phase) Buffer: ACN (60:40 %v/v) to ascertain the final concentrations of 20µg/mL. Ultimately the absorbance was notified at 225 nm for the drug.

5.3.5. Preparation of placebo

The mixture of excipients of marketed formulations of albuterol sulphate MDI as per their standard levels were mixed accurately, except the API, and are subsequently homogenized for half an hour to obtain the placebo. Then the placebo solutions were diluted within the linearity range concentrations and injected in to the UHPLC system.

5.4. Chromatographic conditions

The chromatographic separation was performed in Hibar HR purospher STAR RP-18 encapped column (C18, 250mm × 4.6 mm, 5µm) analytical column which provides competent and rapid separation of analyte based upon its sensitivity of analysis in the UHPLC system. The binary mobile phase composed of (pH 3.0 + 0.05) predominantly the phosphate buffer (10mM KH_2PO_4) and ACN (60:40 %v/v) were employed for the detection. The flow rate was maintained as 1ml/minute, with a volume injection of 10 µL. The column oven temperature was maintained as 40°C during method optimization. The sample temperature is at the optimum condition, monitored as 5°C during the development.

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Common abbreviations: MODR: Method operable design region, UHPLC: Ultra High Performance Liquid Chromatography, CGMP: Cyclic Guanosine Monophosphate, ATP: Analytical Target Profile.

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