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IMPURITY PROFILE AND VALIDATION OF PHARMACEUTICAL (API) BULK DRUG

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ABSTRACT

Pharmaceutical API bulk drug containing three impurities were found during the analysis. High performance liquid chromatography method was developed and validated for the Impurities of albuterol in bulk and capsules dosage forms. The separation was achieved on HPLC Columns (C-104) and (C-118) analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using Acetonitrile (HPLC Grade), Methanol (HPLC Grade) in the ratio 50:50 v/v as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a UV detector at 302nm. The total chromatographic analysis time per sample was about 7.1 min and for impurities 11.6, 12.2 and 12.7. The method was validated for accuracy, linearity, LOD, LOQ and stability of analytical solutions. Validation studies demonstrated that, this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curve was linear over the concentration range of 25-150µg/mL with R_2 close to (0.9998). The limit of detection (LOD) and limit of Quantitation (LOQ) obtained 0.025µg/mL and 0.05µg/mL respectively. The developed and validated method was successfully applied for the quantitative analysis of albuterol capsules. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of albuterol in capsule dosage form.

KEYWORDS: Accuracy, Linearity, LOD, LOQ, HPLC Techniques and Stability in Analytical Solution.

INTRODUCTION

(*RS*)-4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol, also known as albuterol and its branded name as Ventolin along with other some brand names,[1] is a medication that opens up the medium and large airways in the lungs.[2] It is used to treat asthma including asthma attacks, exercise-induced bronchoconstriction and chronic obstructive pulmonary disease. It may also be used to treat high blood potassium levels.[3] Albuterol is usually used with an inhaler or nebulizer but is also available as a pill and intravenous solution.[2,3] Onset of action of the inhaled version is typically within 15 minutes and lasts for two to six hours. Common side effects include shakiness, headache, fast heart rate, dizziness and feeling anxious. Serious side effects may include worsening bronchospasm, irregular heartbeat and low blood potassium levels.[4] It can be used during pregnancy and breastfeeding, but safety is not entirely clear. It is a short-acting β_2 adrenergic receptor agonist which works by causing airway smooth muscles to relax.[5] There are various methods in the literature for the qualitative and quantitative analysis of the Albuterol in the bulk (API) and the pharmaceutical dosage forms. The method was developed and validated under the light of International Conference on Harmonization (ICH) guidelines.[6,7] And for the statistical evaluation of results, standards guidelines were followed.[8, 9] Hence, our aim was to establish an easy and convenient high pressure liquid chromatography (HPLC) technique, which not only useful for researcher but also for the analysts working in the pharmaceutical quality control labs.

MATERIALS AND METHODS

Chemicals & Reagents

The standard bulk drug (API) of Albuterol (99.95% purity) was obtained from Supriya Lifescience Limited, Mumbai. The acetonitrile was in this research by HPLC grade, where other chemicals were high purity with analytical grades. All the chemicals were purchased from Sigma Aldrich.

Apparatus & Chromatographic Conditions

Equipments were used for the validation studies such as HPLC System: SLL/QC/29, 57, Waters 2695 Separation Module, Waters UV & 996 PDA, Empower 2.0 & 3.0 Software, Balance (SLL/QC/50), HPLC Columns (C-104) and (C-118), Photo Stability Chamber (SLL/QC/74) and Hot air oven (SLL/QC/24).

Preparation of Mobile Phase

The preparation of mobile phase solution was prepared by acetonitrile in 50:50 (v/v) ratios. Then, the buffer was mixed with acetonitrile. The final mobile phase was then filtered by passing through 0.5µm membrane filter before uses.

Standard of working albuterol

Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed.

Albuterol Sample (API)

Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed.

RESULTS AND DISCUSSION

Limit of Detection and Limit of Quantification

Based on determination of Prediction linearity, six replicate injections were made for LOD and LOQ. Calibration curves were constructed in a very low concentration region (0.05 to 1.0% of the target concentration) of Albuterol (0.10 to 0.20µg/mL) for the calculation of the limit of detection (LOD) and the limit of quantification (LOQ) using.[10-11] Equations (1) and (2) accordingly.

$LOD = 3.3\ \sigma / S$ -----(1)

$LOQ = 10\sigma/S$ -------(2)

Where σ is the residual standard deviation of the regression line, S is the slope of the standard curve. The LOD and LOQ obtained for albuterol were 0.025µg/mL and 0.05µg/mL, respectively.

Table -1: Limit of detection(LOD).

	Albuterol	**Impurity-A**	**Impurity- B**
Conc. (µg/ml)	**0.1**	**0.1**	**0.1**
Sr.No	Response		
1	631	350	522
2	686	376	506
3	656	355	501
4	644	391	520
5	557	370	508
6	678	378	511
Mean	**642**	**370**	511
SD	**46.43**	**15.27**	8.19
% RSD	**7.230**	**4.130**	1.600

Table-2: Limit of Quantification (LOQ).

	Albuterol	Impurity-A	Impurity-B
Conc. (µg/ml)	**0.2**	**0.2**	**0.2**
Sr.No	Response		
1	1289	717	1155
2	1310	728	1169
3	1290	721	1153
4	1225	706	1124
5	1247	743	1094
6	1299	741	1111
Mean	**1277**	**726**	**1134**
SD	**33.13**	**14.31**	**29.17**
% RSD	**2.590**	**1.970**	**2.570**

Acceptance Criteria**:** RSD for LOD: NMT 33% and RSD for LOQ: NMT 10%.

	Albuterol	Impurity -A	Impurity -B
LOD (% w/w)	0.025	0.025	0.025
Conc. (µg/ml)	0.10	0.10	0.10
LOQ (% w/w)	0.05	0.05	0.05
Conc. (µg/ml)	0.20	0.20	0.20

Linearity

The linearity method was checked by preparing different types of solution of Albuterol from 50% to 200%. Then, a linear regression equation was derived by plotting the graph between the sample dissolved and recovered by the method. From the observation and calculation are given in table 3, it is cleared that the correlation coefficient (R_2) equal to unity and comes under the acceptance criteria ($R_2 \geq 0.999$). Therefore, depending upon calculated values of R2, the developed method should be considered having a high degree of linearity. A series of solutions of Albuterol, Impurity A and Impurity-B were prepared over the range of LOQ to 200% of specification Limit. Acceptance Criteria of the Correlation Coefficient should not be less than 0.99.

Table-3: Linearity of Albuterol, Impurity -A and Impurity-B.

		Albuterol		Impurity-A		Impurity-B	
Sr.No	**Level**	**Conc. (µg/ml)**	**Response (Area)**	**Conc. (µg/ml)**	**Response (Area)**	**Conc. (µg/ml)**	**Response (Area)**
1	LOQ	0.21	1235	0.19	632	0.18	1167
2	Linearity-1	0.32	1957	0.48	1917	0.45	3121
3	Linearity-2	0.40	2287	0.60	2575	0.56	3919
4	Linearity-3	0.48	2905	0.72	3019	0.67	4651
5	Linearity-4	0.64	3709	0.96	4053	0.89	6408
6	Linearity-5	0.80	4723	1.20	5167	1.11	8006
7	Linearity-6	1.19	6956	1.80	7806	1.67	12211
8	Linearity-7	1.59	9356	2.40	10344	2.23	16456
Slope		**5845.14**		**4366.935**		**7409.997**	
Intercept		**-0.0051**		**0.0248**		**0.0236**	
Correlation Coefficient		**0.9998**		**0.9998**		**0.9998**	

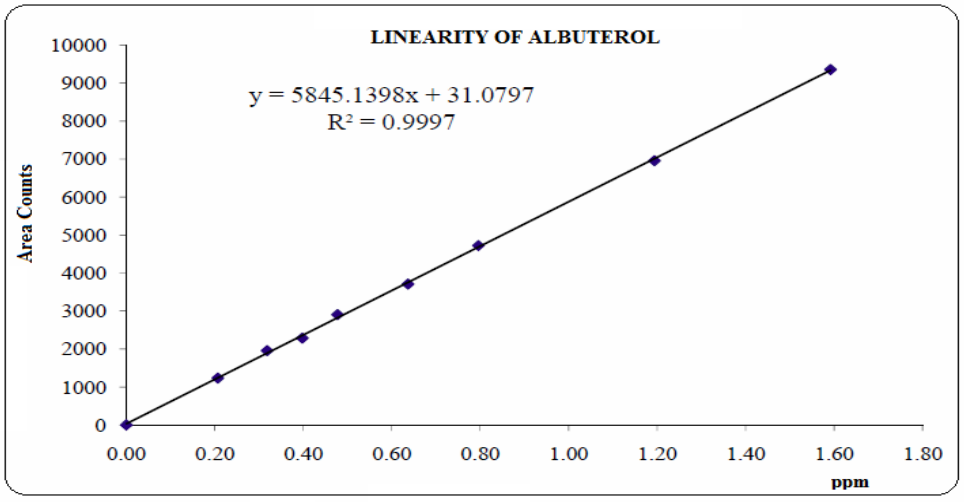

Fig.1: Linearity of Albuterol.

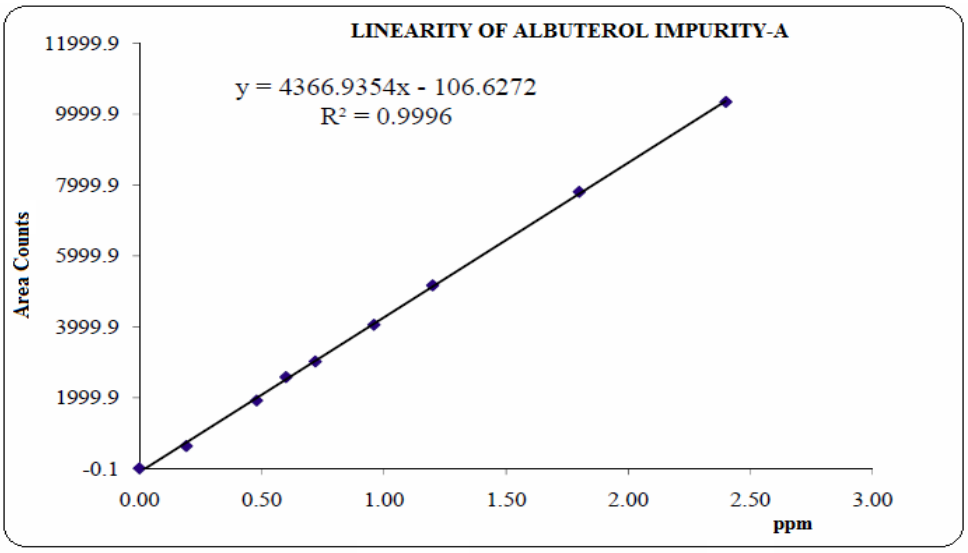

Fig. 2: Linearity of Albuterol Impurity-A.

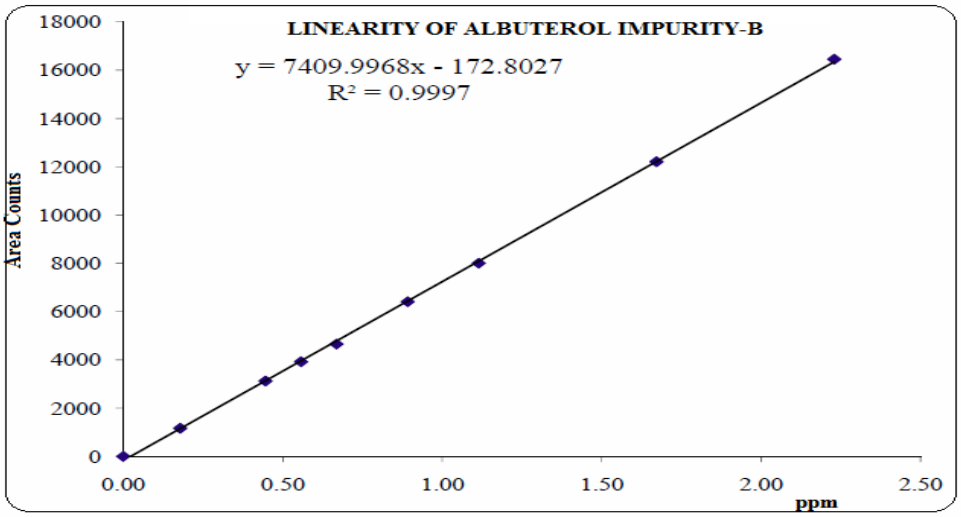

Fig.3: Linearity of Albuterol Impurity-B.

Accuracy

Sample of Albuterol was spiked with known impurities at five different levels: LOQ, 50%, 100%, 150% and 200% of the specification limit in triplicate (total 15 determinations) and then proceed with sample preparation as described under Methodology.[12] The Acceptance Criteria of Mean Recovery should be in the range of 90.0% to 110.0% for 50%, 100%, 150% AND 200% levels. Mean Recovery should be in the range of 70.0% to 130.0% for LOQ levels. The Mean Recovery for known Impurities is within limits. Therefore, the HPLC Method for the determination of Related Substances of albuterol in albuterol API is accurate.

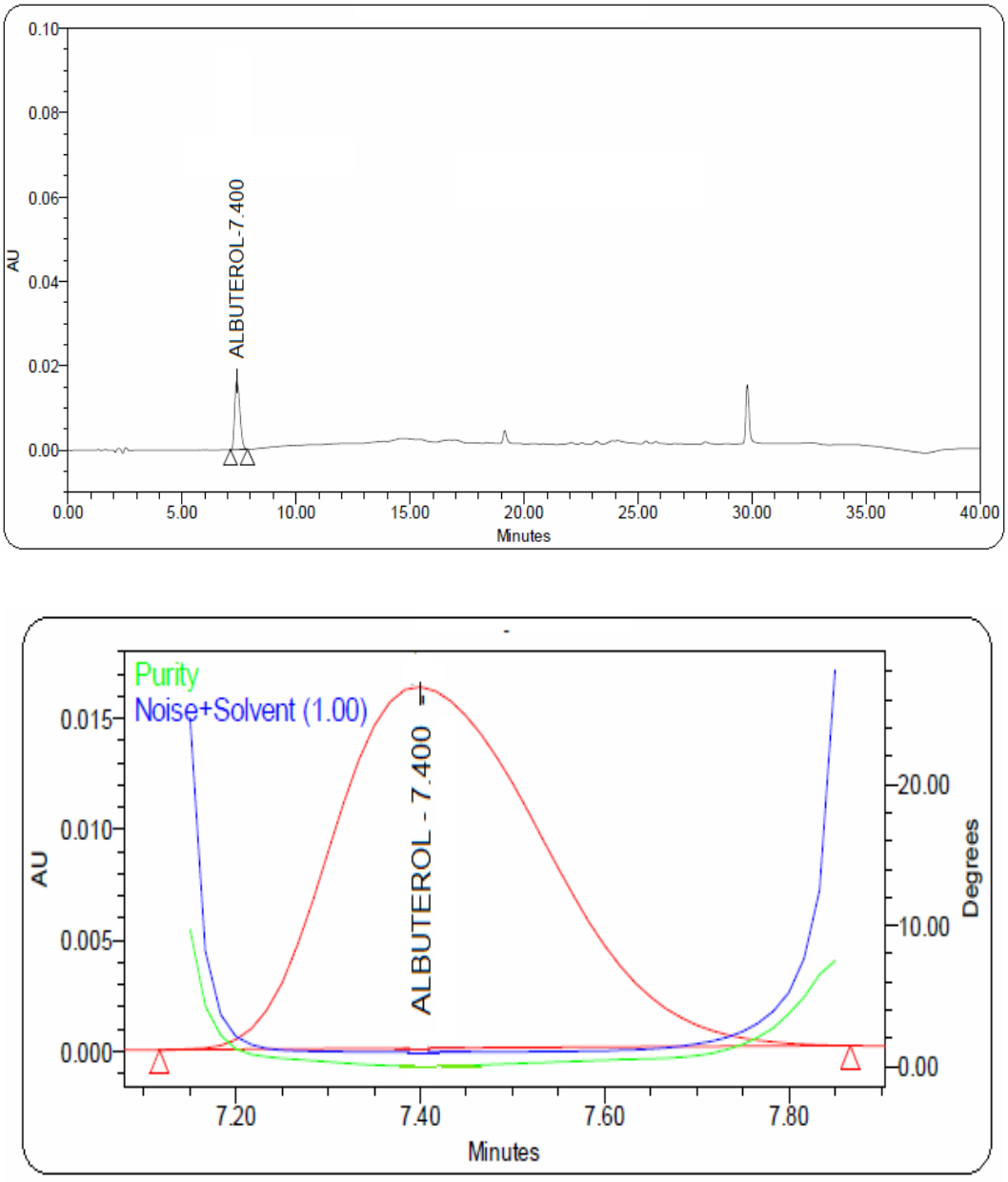

Fig. 4: Chromatogram and Purity Peak of Bulk Drug (API).

Table-4: Recovery Results of Impurity-A.

Recovery Level	Actual Amount Added (mg)	Amount Recovered (mg)	% Recovery
LOQ-1	0.0121	0.013	107.4
LOQ-2	0.0121	0.014	115.7
LOQ-3	0.0121	0.014	115.7
		Mean	**112.9**
		SD	**4.792**
		% RSD	**4.24**
50%-1	0.0300	0.034	113.3
50%-2	0.0300	0.035	116.7
50%-3	0.0300	0.034	113.3
100%-1	0.0610	0.064	104.9
100%-2	0.0610	0.063	103.3
100%-3	0.0610	0.063	103.3
150%-1	0.0910	0.094	103.3
150%-2	0.0910	0.094	103.3
150%-3	0.0910	0.093	102.2
200%-1	0.1210	0.123	101.7
200%-2	0.1210	0.122	100.8
200%-3	0.1210	0.123	101.7
		Mean	**105.7**
		SD	**5.464**
		% RSD	**5.17**

Table-5: Recovery Results of Impurity-B.

Recovery Level	Actual Amount Added (mg)	Amount Recovered (mg)	% Recovery
LOQ-1	0.0089	0.010	112.4
LOQ-2	0.0089	0.010	112.4
LOQ-3	0.0089	0.010	112.4
		Mean	**112.4**
		SD	**0.000**
		% RSD	**0.00**
50%-1	0.028	0.028	100.0
50%-2	0.028	0.028	100.0
50%-3	0.028	0.028	100.0
100%-1	0.056	0.057	101.8
100%-2	0.056	0.057	101.8
100%-3	0.056	0.057	101.8
150%-1	0.084	0.086	102.4
150%-2	0.084	0.086	102.4
150%-3	0.084	0.086	102.4
200%-1	0.110	0.115	103.6
200%-2	0.110	0.114	102.7
200%-3	0.110	0.114	102.7
		Mean	**101.8**
		SD	**1.193**
		% RSD	**1.17**

Stability in Analytical Solution

Sample solutions of Albuterol and sample solutions of Albuterol spiked with Impurities A and B were prepared analyzed initially and at different intervals of time at room temperature and the results were recorded accordingly. The Acceptance Criteria of Cumulative % RSD should not be more than 10%. The % Cumulative RSD is within limits. Therefore Impurities in sample solutions are stable for 24 hours at room temperature. Reference Solutions are stable up to 49 hours.

Table -6: Solution Stability of Sample Solution (As Such).

	Impurity- C		
Time (In hrs)	**Area**	**Mean**	**Cumulative % RSD**
Initial	3489		
12:15	3476	3483	0.26
13:01	3483	3483	0.19
13:47	3451	3475	0.48
14:33	3493	3478	0.48
15:19	3448	3473	0.56
16:05	3501	3477	0.59
16:51	3502	3480	0.60
17:36	3496	3482	0.58
27:34	3449	3479	0.63
28:20	3468	3478	0.60
29:06	3490	3479	0.58
29:52	3478	3479	0.56
30:37	3491	3480	0.54
31:23	3464	3479	0.54
32:09	3479	3479	0.52
32:55	3452	3477	0.54

Table-7: Solution Stability of Spiked Solution.

	Impurity C			Impurity-A			Impurity-B		
Time (In hrs)	**Area**	**Mean**	**RSD***	**Area**	**Mean**	**RSD***	**Area**	**Mean**	**RSD***
Initial	3245			4516			8368		
19:08	3246	3246	0.02	4450	4483	1.04	8294	8331	0.63
19:54	3207	3233	0.69	4448	4471	0.87	8386	8349	0.58
20:40	3225	3231	0.57	4408	4456	1.00	8309	8339	0.54
21:26	3223	3229	0.51	4433	4451	0.90	8312	8334	0.49
22:12	3209	3226	0.52	4417	4445	0.86	8289	8326	0.49
22:58	3207	3223	0.53	4434	4444	0.79	8356	8331	0.46
23:44	3217	3222	0.49	4427	4442	0.75	8320	8329	0.43
24:30	3208	3221	0.48	4424	4440	0.71	8287	8325	0.44
34:27	3233	3222	0.47	4364	4432	0.86	8268	8319	0.47
35:13	3200	3220	0.49	4347	4424	1.00	8260	8314	0.49
35:59	3231	3221	0.48	4385	4421	0.99	8334	8315	0.47
36:45	3159	3216	0.70	4373	4417	1.00	8277	8312	0.47
37:31	3251	3219	0.74	4369	4414	1.00	8228	8306	0.53
38:17	3235	3220	0.72	4395	4413	0.97	8246	8302	0.54
39:03	3240	3221	0.71	4395	4412	0.95	8302	8302	0.52
39:49	3189	3219	0.73	4364	4409	0.95	8233	8298	0.55

*Cumulative % RSD.

Table-8: Solution Stability of Reference Solutions.

Reference Solution (a)							Reference Solution (b)			
Time (In hrs)	Impurity-A			Impurity- B			Time (In hrs)	Albuterol		
	Area	Mean	RSD*	Area	Mean	RSD*		Area	Mean	RSD*
Initial	4820			6894			Initial	2663		
0:45	4829	4825	0.13	6879	6887	0.15	0:45	2755	2709	2.40
1:31	4723	4791	1.23	6892	6888	0.12	1:31	2751	2723	1.91
2:17	4768	4785	1.03	6867	6883	0.18	2:17	2722	2723	1.56
3:03	4762	4780	0.92	6875	6881	0.17	3:03	2806	2739	1.91
3:49	4793	4783	0.83	6842	6875	0.28	3:49	2755	2742	1.72
17:36	4735	4776	0.85	6985	6891	0.65	13:46	2756	2744	1.58
22:58	4675	4763	1.08	6975	6901	0.74	19:08	2788	2750	1.57
30:37	4708	4757	1.09	6968	6909	0.77	26:48	2785	2753	1.53
38:17	4688	4750	1.12	7026	6920	0.90	34:27	2859	2764	1.87
45:56	4642	4740	1.27	7038	6931	0.99	42:06	2858	2773	2.05
53:36	4654	4733	1.32	7059	6942	1.09	49:46	2900	2783	2.35

*Cumulative % RSD.

CONCLUSIONS

The HPLC method for the determination of Related Substances of albuterol in albuterol API is Robust for a small change in Wavelength, Flow and Column temperature, except a small change in Mobile Phase pH and Flow Plus. The Mean Recovery for known Impurities is within limits. Therefore, the HPLC Method for the determination of Related Substances of albuterol in albuterol API is accurate. The correlation coefficient for albuterol, Impurity -A and Impurity-B is more than 0.99. Therefore, the HPLC Method for the determination of related Substances of albuterol in albuterol API is Linear. The % Cumulative RSD is within limits. Therefore Impurities in sample solutions are stable for 24 hours at room temperature. Reference Solutions are stable up to 49 hours.

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