IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 11, Issue 2 Ver. II (February. 2018), PP 01-20
www.iosrjournals.org

Validation of Pharmaceutical (API) Bulk Drug by HPLC Methods

Sushama R. Ambadekar[1], Iyer Balakrishnan[2], Manohar V. Lokhande[3*]
[1,2]*Department of Chemistry, Institute of Science, Mumbai 400 032, Maharashtra State, India*
[3*]*Department of Chemistry, Sathaye College, Mumbai-400057, Maharashtra state, India.*
Corresponding Author : Manohar Lokhande

***Abstract*:** *Salbutamol (API) belongs to the class of bronchodilators. It is used to treat asthma, chronic bronchitis and other breathing disorders. Salbutamol (API) having two impurities have been identified by HPLC method. These impurities are process related and batch process related impurities. These impurities are found by two different chromatograms isolated by HPLC method. The impurities are not more than 0.3% and unspecified impurities are not more than 0.1%. These impurities were identified by using HPLC system; SLL/QC/29, 57 using columns (C-104 & C- 118), photo stability chamber (SLL/QC/74). From the experimental data of LOD & LOQ, the mean values of impurities A & B are 370 & 511 whereas the standard value of drug is 642. SD values of impurities A & B are 15.27 & 8.19 whereas the standard SD value of drug is 46.43. The percentage RSD value for standard drug is 7.230% and impurities A & B are 4.130 & 1.6%. We have also calculated some parameters for validation of drug like specificity, linearity, accuracy, precision and system suitability.*

***Key Words*:** *Salbutamol (API), HPLC method, linearity, Forced Degradation Studies, System suitability, precision and accuracy.*

Date of Submission: 26-01-2018 Date of acceptance: 13-02-2018

I. Introduction

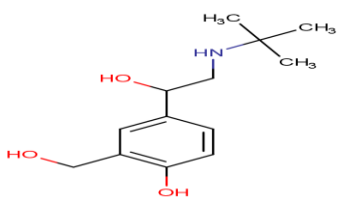
Salbutamol is used to treat or prevent bronchospasm in patients with asthma, bronchitis, emphysema & other lung diseases. This medicine is also used to prevent wheezing caused by exercise (exercise-induced bronchospasm). Salbutamol belongs to the family of medicines known as adrenergic bronchodilators. Adrenergic bronchodilators are medicines that are breathed in through mouth to open up bronchial tubes (air passages) in the lungs. They relieve cough, wheezing, shortness of breath, and troubled breathing by increasing the flow of air through the bronchial tubes. Salbutamol a moderately selective beta (2)-receptor agonist similar in structure to terbutaline, is widely used as a bronchodilator to manage asthma and other chronic obstructive airway diseases. The R-isomer, levalbuterol, is responsible for bronchodilation while the S-isomer increases bronchial reactivity. The R-enantiomer is sold in its pure form as Levalbuterol. The manufacturer of levalbuterol, Sepracor, has implied (although not directly claimed) that presence of only the R-enantiomer produces fewer side-effects [1,2].

The challenge of different types of pharmaceutical industry is to produce quality product. it is necessary to check the purity and quality control checks to maintain quality and purity of the bulk drugs in every pharmaceutical industry. Raw materials, manufacturing method, crystallization and purification process play an important role to maintain the purity of the product. Analytical chemistry which is related to the developmental concepts in industry also changes with time [3,4]. Stringent limits of purity and impurity is specified by various pharmacopoeias. Modern separation methods are advanced as these methods simultaneously separate, quantify components to make separation and characterization of impurities easier. Salbutamol bulk drug (API) and in pharmaceutical dosage forms. The methods were developed and validated [5,6] under guidelines of International Conference on Harmonization (ICH) [7,8] for statistical evaluation of results, standards guidelines were followed. 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 analysts working in pharmaceutical quality control labs [9]. In this research paper, we have also calculated some parameters for validation of drug like specificity, linearity, accuracy, precision and system suitability [10].

Molecular formula: $C_{13}H_{21}NO_3$ Molecular Weight: 239.315g/mol

IUPAC Name: 4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol

***Structure of Salbutamol*:**



***Appearance*:** White or almost white, crystalline powder.
***Solubility*:** Freely soluble in water, practically insoluble or very slightly Soluble in ethanol (96 %t) and in Methylene chloride.

II. Experimental

***Instruments and Reagents*:** Following Equipments were used for the validation studies.
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); Hot air oven (SLL/QC/24).
Chemicals: Acetonitrile (HPLC Grade): Methanol (HPLC Grade): Sodium Dihydrogen phosphate monohydrate (AR grade): Triethylamine (AR Grade): Ortho Phosphoric acid (HPLC Grade) : Water for HPLC .
Standard: Salbutamol working standard: Use of standard & use % potency on as is basis for calculations. Keep container tightly closed. Potency: 99.5 % w/w on as is basis.
Salbutamol Sample (API): Use of standard as such& use % potency on as is basis for calculations. Keep container tightly closed. Potency: 100.0 % w/w on as is basis

I. METHODOLOGY:

<table>
<tr><td colspan="3">Related substance by HPLC (%) : EP Method</td></tr>
<tr><td colspan="3">Specification:
Impurity D : Not more than 0.3% w/w
Impurity F : Not more than 0.3% w/w
Impurity C : Not more than 0.2% w/w
Impurity N : Not more than 0.2% w/w
Impurity O : Not more than 0.2% w/w
Unspecified Impurity : Not more than 0.10% w/w
Total Impurities : Not more than 0.9% w/w
Disregard limit : Not more than 0.05% w/w</td></tr>
<tr><td colspan="3">Procedure:</td></tr>
<tr><td>Test Solution</td><td colspan="2">Weigh accurately about 20.0 mg of sample into the mobile phase A and dilute to 50.0 mL with the mobile phase A.</td></tr>
<tr><td>Preparation of Mobile Phase B</td><td colspan="2">Methanol: Acetonitrile (35: 65 V/V)</td></tr>
<tr><td>Gradient Programme</td><td>Time (Min)
00
05
30
35
40
45</td><td>Mobile Phase A (% V/V)
95
95
10
10
95
95</td><td>Mobile Phase B (% V/V)
05
05
90
90
05
05</td></tr>
<tr><td>Identification of impurities</td><td colspan="2">1. Use the chromatogram supplied with Salbutamol for system suitability and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities C, D, F, N and O.
2. Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity J.</td></tr>
<tr><td>In the system suitability test for reference solution</td><td colspan="2">A. Peak to Valley Ratio:
1. Not less than 1.2, where height (Hp) above baseline of peak due to impurity- D & height (Hv) above baseline of lowest point of curve separating this peak from peak due to Impurity N in chromatogram obtained with Reference solution (e).
2.Not not less than 2.0 where Hp = height above baseline of peak due to impurity J and H, = height above baseline of lowest point of curve separating this peak from peak due to Salbutamol in chromatogram obtained with reference solution (c).
B.% RSD:
% RSD of Area Counts not more than 5.0 for peaks due to Impurity-D and Impurity-F in chromatograms obtained with replicate injections of Reference solution (a) and for peak due to Salbutamol in the Reference Solution (b).</td></tr>
<tr><td colspan="3"></td></tr>
</table>

Calculation:

$$\text{Impurity D : (\% w/w)} = \frac{AT_1}{AS_1} \times \frac{\text{Std wt (mg)}}{50} \times \frac{2}{100} \times \frac{50}{\text{Spl. Wt (mg)}} \times \frac{P_1}{100} \times 100$$

$$\text{Impurity F : (\% w/w)} = \frac{AT_2}{AS_2} \times \frac{\text{Std wt (mg)}}{50} \times \frac{2}{100} \times \frac{50}{\text{Spl. Wt (mg)}} \times \frac{P_2}{100} \times 100$$

$$\text{Impurity C : (\% w/w)} = \frac{AT_3}{AS_3} \times \frac{\text{Spl wt (mg)}}{50} \times \frac{1}{100} \times \frac{1}{10} \times \frac{50}{\text{Spl. Wt (mg)}} \times \frac{P_3}{100} \times 100$$

$$\text{Impurity N : (\% w/w)} = \frac{AT_4}{AS_3} \times \frac{\text{Spl wt (mg)}}{50} \times \frac{1}{100} \times \frac{1}{10} \times \frac{50}{\text{Spl. Wt (mg)}} \times \frac{P_3}{100} \times 100$$

$$\text{Impurity O : (\% w/w)} = \frac{AT_5}{AS_3} \times \frac{\text{Spl wt (mg)}}{50} \times \frac{1}{100} \times \frac{1}{10} \times \frac{50}{\text{Spl. Wt (mg)}} \times \frac{P_3}{100} \times 100$$

$$\text{Any Unspecified : (\% w/w)} = \frac{AT_6}{AS_3} \times \frac{\text{Spl wt (mg)}}{50} \times \frac{1}{100} \times \frac{1}{10} \times \frac{50}{\text{Spl. Wt (mg)}} \times \frac{P_3}{100} \times 100$$

Total impurities = Impurity D + F + C + N + O + Sum of Unspecified impurities.

Where,

AT_1 = Area Counts of peak due to impurity- D in Test chromatogram.
AT_2 = Area Counts of peak due to impurity- F in Test chromatogram.
AT_3 = Area Counts of peak due to impurity- C in Test chromatogram.
AT_4 = Area Counts of peak due to impurity- N in Test chromatogram.
AT_5 = Area Counts of peak due to impurity- O in Test chromatogram.
AT_6 = Area Counts of peak due to Unspecified impurity in Test chromatogram.
AS_1 = Average Area Counts of peak due to impurity- D in chromatogram reference Solution (a).
AS_2 = Average Area Counts of peak due to Impurity- F in chromatogram reference Solution (a).
AS_3 = Average Area Counts of peak due to Salbutamol in chromatogram reference Solution (b).
P_1 = % Potency of Impurity- D working standard on as is basis.
P_2 = % Potency of Impurity- F working standard on as is basis.
P_3 = % Potency of Salbutamol working standard on as is basis.

III. Result and Discussion

***Specificity*:**

Identification: It is the ability to measure desired analyze in a complex mixture. Specificity is the ability to assess unequivocally the target pathogen or analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Individual impurities of Salbutamol Impurity-D,J and F are injected to check the Retention times along with and Salbutamol for System Suitability (containing impurities C, F, N and O). Salbutamol standard and Sample are also prepared and injected in HPLC using chromatographic system described in the Methodology by using a photodiode array detector. *Acceptance Criteria*: Retention time of Salbutamol and known impurities in standard and sample should be comparable. Peak purity should pass for Salbutamol and known impurities in control sample and spiked sample. The retention times are comparable for Salbutamol and its known Impurities. Peak purity passes for Analyte peak. Hence the method is Selective **(See table -1&2, Figure 1-7)**

Accuracy: It is the agreement between measured and real value. The closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value, and the value found. Accuracy is measure of experimental values. Accuracy studies for drug substance & drug product are recommended to be performed at 80, 100 and 120% levels, the guideline for submitting samples, analytical data for methods validation. For drug product, this is performed frequently by addition of known amounts of drug by weight or volume (dissolved in diluents) to formulation working in linear range of detection of analyte.

Sample of Salbutamol 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. *Acceptance Criteria:* Mean Recovery is in range of 90.0 % to 110.0% for 50 %, 100 % , 150 % AND 200% levels. Mean Recovery should be in range of 70.0 % to 130.0% for LOQ levels. The Mean Recovery for known Impurities is within limits. Thus, HPLC Method for determination of Related Substances of Salbutamol in Salbutamol API is accurate. **(See table -3&4)**

Precision: It is the agreement between a series of measurements. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic (full scale) samples. However, if it is not possible to obtain a full-scale sample it may be investigated using a pilot-scale or bench-top scale sample or sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Detection Limit The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

System Precision**:** Experiment: Six replicate injections of the standard preparation were made into the HPLC using the method described under Methodology. *Acceptance Criteria***:** RSD values are not more than 5.0% . % RSD for known impurities and Salbutamol are well within limits. Thus, HPLC Method for determination of related substances of Salbutamol in Salbutamol API is Precise. **(See table -5)**

Method Precision**:** Six sample preparations of Salbutamol were prepared as specified in Methodology and also by spiking with known concentrations (at specification limit concentrations) of Impurity- D, Impurity -F and injected into HPLC using method as described under Methodology. **(See table -6 &7)**

Linearity: It is the proportionality of measured value to concentration. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Note: Measurements using clean standard preparations should be performed to demonstrate detector linearity, while method linearity should be determined concurrently during the accuracy study. A series of solutions of Salbutamol, Impurity D and Impurity F were prepared over the range of LOQ to 200 % of specification Limit. *Acceptance Criteria***:** Correlation Coefficient is not less than 0.99. The correlation coefficient for Salbutamol, Impurity- D and Impurity -F is more than 0.99. Thus, HPLC Method for determination of related Substances of Salbutamol in Salbutamol API is Linear. **(See table -8, Figure-8)**

System suitability: In addition, prior to start of laboratory studies to demonstrate method validity, some type of system suitability must be done to demonstrate that analytical system is performing properly. Examples include: replicate injections of a standard preparation for HPLC and GC methods; standardization of a volumetric solution followed by assays using the same burette for titrimetric methods; replicate scanning of same standard preparation during UV-VIS assays, etc. When method in question utilizes an automated system such as a chromatograph or an atomic absorption spectrophotometer, a suitable standard preparation should be intermittently measured during sample analysis run. The responses generated by the standard should exhibit a reasonable relative standard deviation. This is done primarily to demonstrate stability of system during sample measurements. System suitability for dissolution studies should be performed using both USP non-disintegrating and disintegrating tablets prior to the validation of dissolution methods. Recorded the resolution between Impurity B and Salbutamol at all experiments

*Acceptance Criteria***:** ***a) Peak to Valley Ratio: 1)***Minimum 1.2, where Hp = height above the baseline of the peak due to impurity N and H, = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity D in the chromatogram obtained with reference solution (e). 2) Minimum 2.0, where Hp = height above the baseline of the peak due to impurity J and H, = height above the baseline of the lowest point of the curve separating this peak from the peak due to Salbutamol in the chromatogram obtained with reference solution (c). ***b) % RSD*****:** 1) Should not be More than 5.0 for the peaks due to Impurity D and Impurity F in the chromatograms obtained with replicate injections of reference solution (a). 2) Should not be More than 5.0 for the peaks due to Salbutamol in the chromatograms obtained with replicate injections of reference solution (b). **(See table -9, Figure-8)**

Forced Degradation Studies:

a)Acid Degradation (5N HCl): Weighed accurately 20 mg of the sample in a 50ml volumetric flask, added 5ml of Mobile Phase- A and sonicated to dissolve. Added 5ml of 5N HCl and heated at 70°C for 3 hours on a water bath. Removed flask from water bath allowed flask to cool at room temperature. Added 5ml of 5N NaOH to neutralize the solution. Cooled at room temperature and diluted to volume with Mobile Phase- A and mixed.(**Figure-9& 10)**

*b)Base Degradation (5N NaOH):*Weighed accurately 20 mg of sample in a 50ml volumetric flask, added 5ml of Mobile Phase- A and sonicated to dissolve. Added 5ml of 5N NaOH and heated at 70°C for 3 hours on a water bath. Removed flask from water bath allowed flask to cool at room temperature. Added 5ml of 5N HCl to neutralize solution. Cooled to room temperature and diluted to volume with Mobile Phase- A and mixed. .(**Figure-11&12)**

c) Peroxide Degradation (30% H_2O_2): Weighed accurately 20 mg of the sample in a 50ml volumetric flask, added 5ml of Mobile Phase- A and sonicated to dissolve. Added 5ml of 30% v/v H_2O_2 and heated at 70°C for 3 hours on a water bath. Removed flask from water bath allowed flask to cool at room temperature. Diluted to volume with Mobile Phase -A and mixed.(**Figure-13&14)**

*d)Reduction Degradation (10% Sodium Bisulphate)***:**Weighed accurately 20 mg of sample in a 50ml volumetric flask, added 5ml of Mobile Phase- A sonicated to dissolve. Added 5ml of 10% w/v sodium Bisulphate and heated at 70°C for 3 hours on a water bath. Removed flask from water bath allowed the flask to cool at room temperature. Diluted to volume with Mobile Phase -A and mixed.(**Figure-15 &16)**

*e) Hydrolysis Degradation***:** Weighed accurately 20 mg of the sample in a 50ml volumetric flask, added 10ml of water and sonicated to disperse and dissolve and heated at 70°C for 3 hours on a water bath. Removed flask from water bath, allowed flask to cool at room temperature and diluted to volume with Mobile Phase -A and mixed.(**Figure-17 &18)**

*f) Heat Degradation***:** Weighed accurately 20 mg of the sample in a 50ml volumetric flask, added 30ml of Mobile Phase A and sonicated to disperse and dissolve and heated at 70°C for 3 hours on a water bath. Removed flask from water bath, allowed flask to cool at room temperature and diluted to volume with Mobile Phase A and mixed.(**Figure-19)**

g) Thermal Degradation (80°C / 72 hrs): Sample was exposed at 80°C for 72 hrs and analyzed to exposed sample as per Methodology.(**Figure-20&21)**

h) Humidity Degradation (25°C / 92% RH for 72 hrs): Sample was exposed at 25°C / 92% RH for 72 hrs and analyzed to exposed sample as per Methodology.(**Figure-22& 23)**

i)Photolytic Degradation (1.2 Million lux hours): Sample was exposed to 1.2 Million lux hours of light and analyzed to exposed sample as per Methodology.(**Figure-24 &25)**

.IV. Tables and Figures

Table.1 Identification by Retention Time

Name	Retention Time (Min)
Salbutamol	7.164
Impurity C	11.604
Impurity D	12.263
Impurity F	12.738
Impurity J	6.772
Impurity N	11.707
Impurity O	13.265

Table.2 Peak Purity of Standards and Control Sample (Diluted)

Sample	Salbutamol	
	Purity Angle	Purity Threshold
Impurity J	2.758	4.486
Impurity F	0.515	2.007
Salbutamol Standard	0.235	2.811
Sample Solution	0.204	1.059

Table-3: Recovery Results of Impurity D

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-4: Recovery Results of Impurity- F

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**

Table-5: System Precision

	Reference Solution- A		Reference Solution- B
# Injection	Impurity-D	Impurity- F	Salbutamol
1	4820	6894	2663
2	4829	6879	2806
3	4723	6892	2755
4	4768	6867	2751
5	4762	6875	2755
6	4793	6842	2722
Mean	**4783**	**6875**	**2742**
SD	**39.62**	**19.07**	**42.43**
% RSD	**0.828**	**0.277**	**1.547**

Table-6: Method Precision (As Such)

Sample	Impurity- D	Impurity -F	Impurity -C	Highest Unspecified	Total RS
Method Precision - 1	ND	ND	0.13	0.07	0.28
Method Precision - 2	ND	ND	0.13	0.07	0.28
Method Precision - 3	ND	ND	0.13	0.07	0.28
Method Precision - 4	ND	ND	0.13	0.07	0.28
Method Precision - 5	ND	ND	0.13	0.07	0.25
Method Precision - 6	ND	ND	0.13	0.07	0.28
Mean			**0.00**		**0.28**
SD			**0.000**		**0.012**
% RSD			**0.00**		**4.45**

Table-7: Method Precision (Spiked)

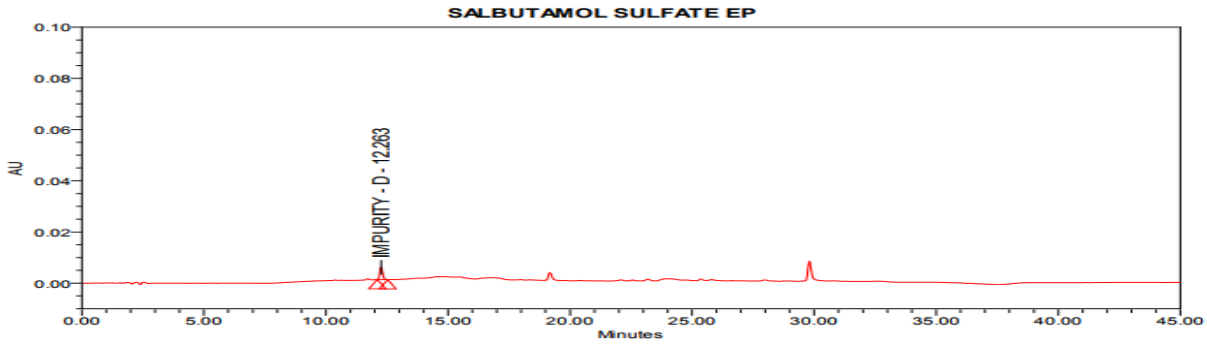
Sample	Impurity- D	Impurity -F	Impurity- C	Highest Unspecified	Total RS
Method Precision - 1	0.28	0.34	0.12	0.08	0.91
Method Precision - 2	0.28	0.34	0.12	0.07	0.92
Method Precision - 3	0.28	0.34	0.12	0.07	0.89
Method Precision - 4	0.28	0.33	0.12	0.08	0.90
Method Precision - 5	0.28	0.33	0.12	0.07	0.89
Method Precision - 6	0.28	0.34	0.12	0.07	0.90
Mean	**0.28**	**0.34**	**0.12**		**0.90**
SD	**0.000**	**0.005**	**0.000**		**0.012**
% RSD	**0.00**	**1.53**	**0.00**		**1.30**

Table- 8: Linearity of Salbutamol, Impurity- D and Impurity -F

	Salbutamol		**Impurity- D**		**Impurity- F**	
Level	Concn (µg/ml)	Response (Area)	Concn (µg/ml)	Response (Area)	Concn (µg/ml)	Response (Area)
LOQ	0.21	1235	0.19	632	0.18	1167
Linearity-1	0.32	1957	0.48	1917	0.45	3121
Linearity-2	0.40	2287	0.60	2575	0.56	3919
Linearity-3	0.48	2905	0.72	3019	0.67	4651
Linearity-4	0.64	3709	0.96	4053	0.89	6408
Linearity-5	0.80	4723	1.20	5167	1.11	8006
Linearity-6	1.19	6956	1.80	7806	1.67	12211
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**	

Table-9: System Suitability

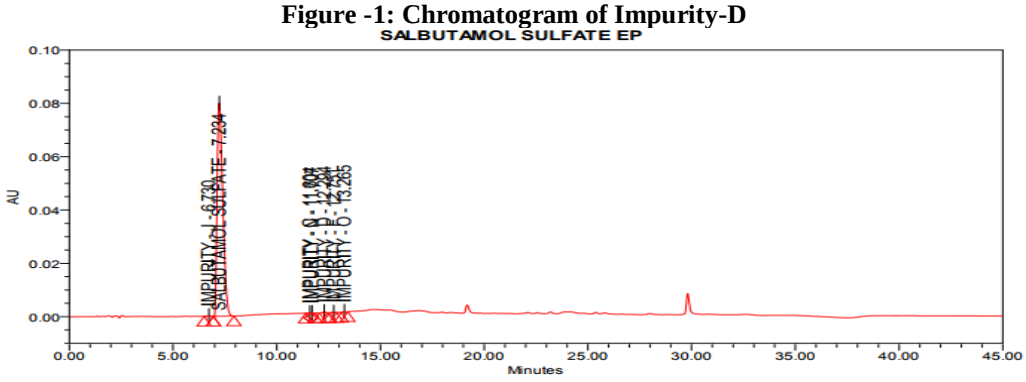
Parameter	Peak to Valley Ratio		Reference Solution (a)		Reference Solution (b)
	Impurity N / D (Ref. sol. e)	Impurity J / Salbutamol (Ref. sol. c)	% RSD of Imp -D	% RSD of Imp- F	% RSD of Salbutamol
Limits	Min. 1.2	Min 2.0	NMT 5.0	NMT 5.0	NMT 5.0
System Precision	>2.0	1.7	0.83	0.28	1.73
Method Precision					
Prediction Linearity	>2.0	15.7	0.63	0.32	0.94
	>2.0	21.2	0.78	0.40	0.98
Linearity	>2.0	3.8	1.14	0.25	1.08
Accuracy	>2.0	22.3	1.18	0.32	0.79
Specificity	>2.0	4.78	4.0	1.47	2.67
Forced Degradation					
Intermediate Precision	>2.0	2.0	1.18	0.32	9.4



Peak results

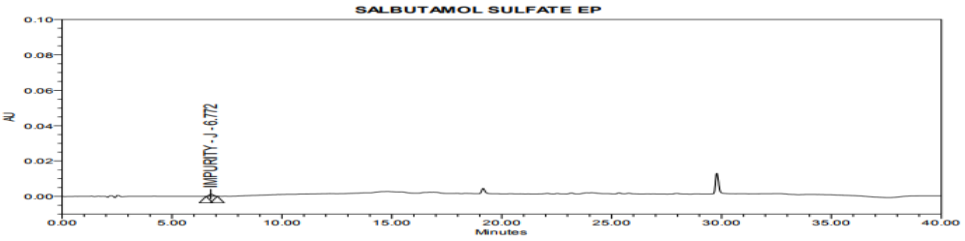
Name	RT	Area	% Area	USP Resolution	Tailing	End p/v
IMPURITY - D	12.263	40475	100.0	48619	1.16	

Figure -1: Chromatogram of Impurity-D



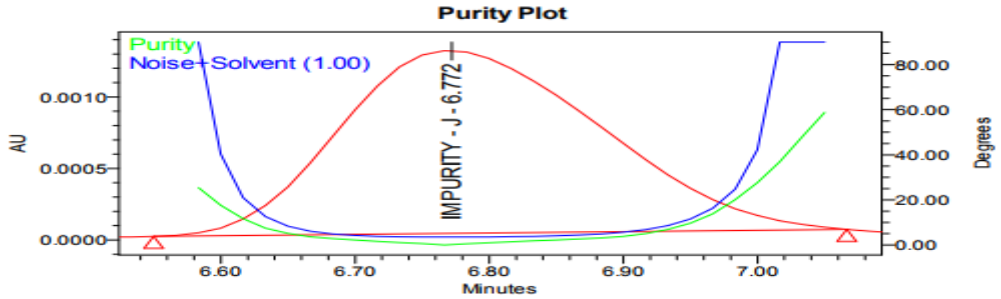
Name	RT	Area	% Area	RT Ratio	USP Plate Count	USP Resolution	Tailing	End p/v
IMPURITY - J	6.730	0.20	2999	0.93	6200		1.06	
SALBUTAMOL SULFATE	7.234	1512618	99.23		3202	1.16	1.51	
IMPURITY - C	11.604	1151	0.08	1.60				1.2
IMPURITY - N	11.707	909	0.06	1.62				
IMPURITY - D	12.294	3327	0.22	1.70	41233		0.84	
IMPURITY - F	12.751	1655	0.11	1.76	20519	1.71	0.74	
IMPURITY - O	13.265	1716	0.11	1.83	44325	1.89	0.90	

Figure-2: Chromatogram of Salbutamol for System Suitability



Peak results

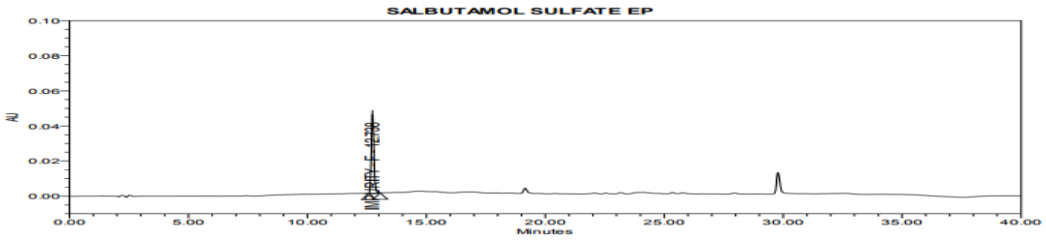
Name	RT	Area	% Area
IMPURITY - J	6.772	17175	100.00



Peak results

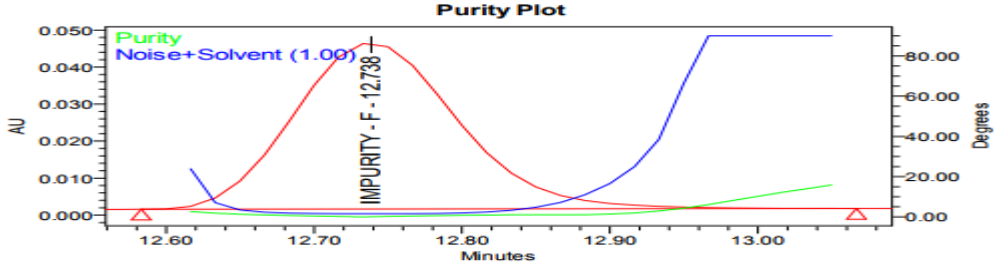
Impurity-J	Purity angle	Threshold
6.772	2.758	4.485

Figure-3: Chromatogram and Peak Purity of Impurity- J



Peak results

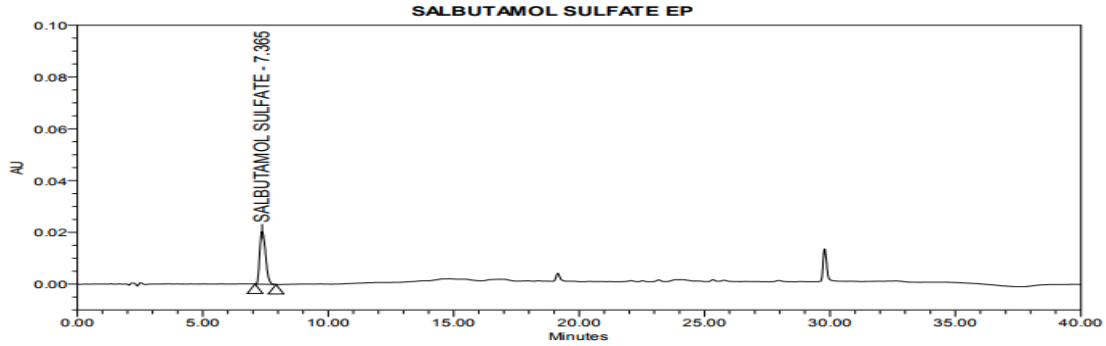
Name	RT	Area	% Area
IMPURITY - F	12.738	346011	100.0



Peak results

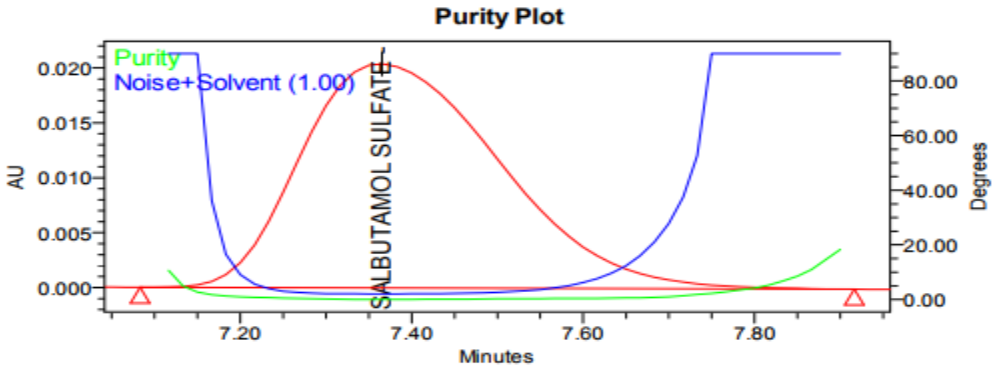
Impurity-F	Purity angle	Threshold
12.738	0515	2.002

Figure-4: Chromatogram and Peak Purity of Impurity- F



Peak results

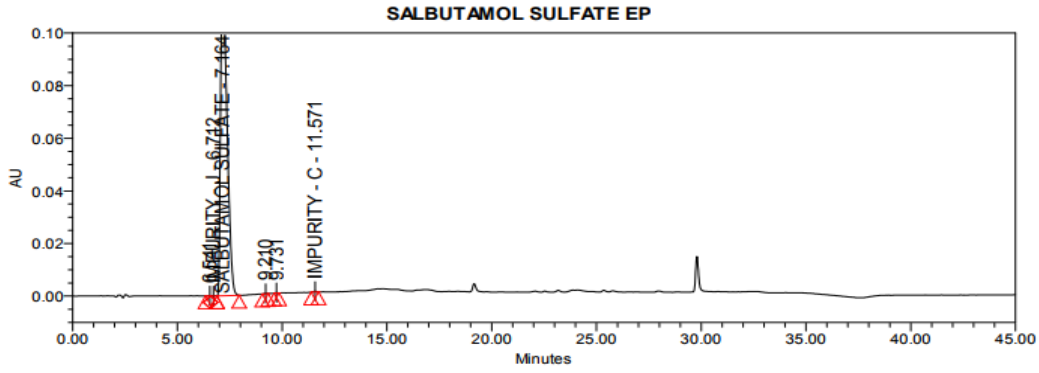
Name	RT	Area	% Area
SALBUTAMOL SULFATE	7.365	329526	100.0



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.235	2.811

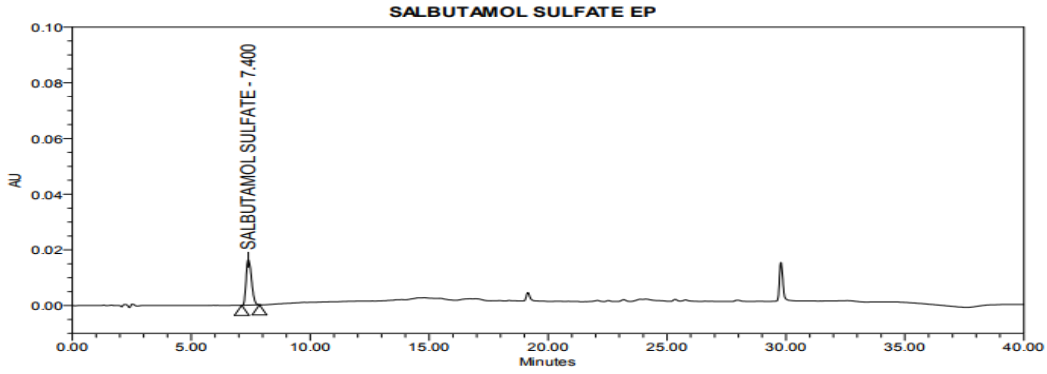
Figure-5: Chromatogram and Peak Purity of Salbutamol



Peak results

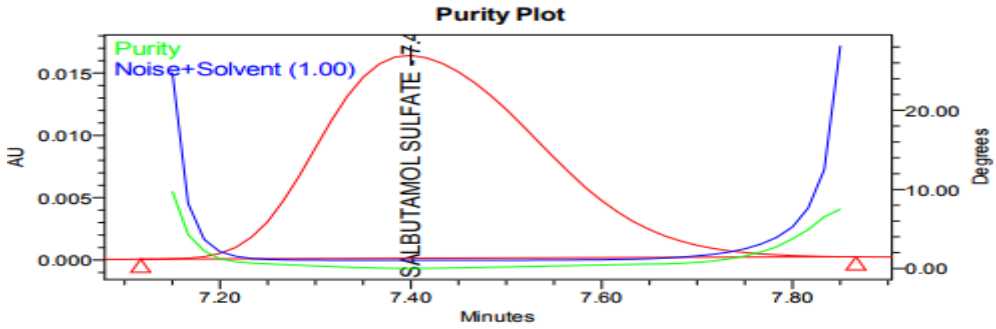
Name	RT	Area	% Area	RT Ratio
	6.541	768	0.03	
IMPURITY - J	6.695	1368	0.05	0.94
SALBUTAMOL SULFATE	7.164	2585224	99.70	
		1040	0.04	
		1421	0.05	
IMPURITY - C	11.571	3109 0	0.12	1.62

Figure-6: Chromatogram of Salbutamol Control Sample



Peak results

Name	RT	Area	% Area
SALBUTAMOL SULFATE	7.400	258923	100.00



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
7.438	0.204	1.059

Figure-7: Chromatogram and peak purity of Control Sample (Diluted)

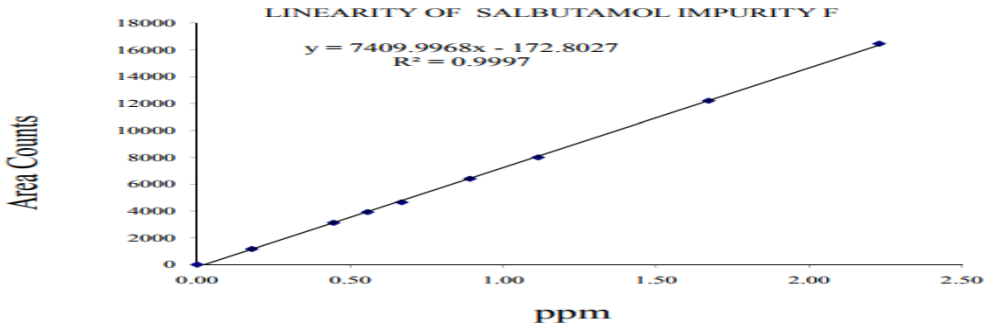
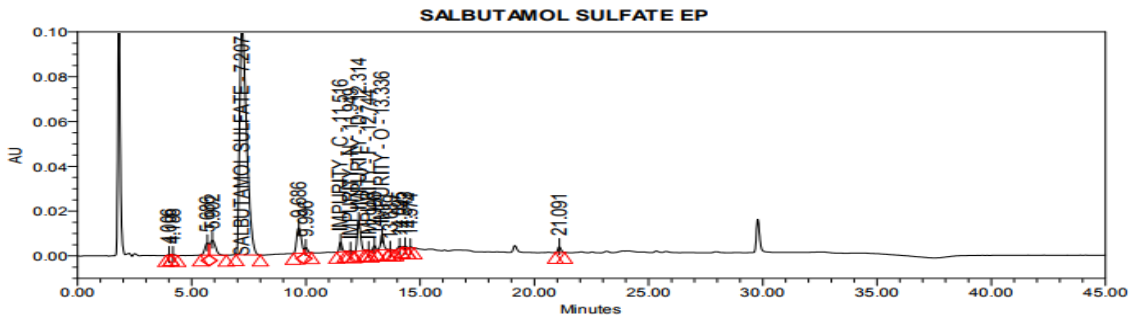


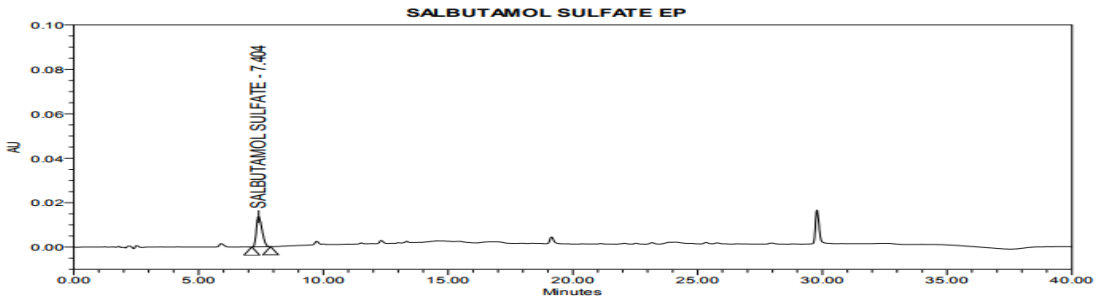
Figure-8: linearity of Impurity-F



Peak Results

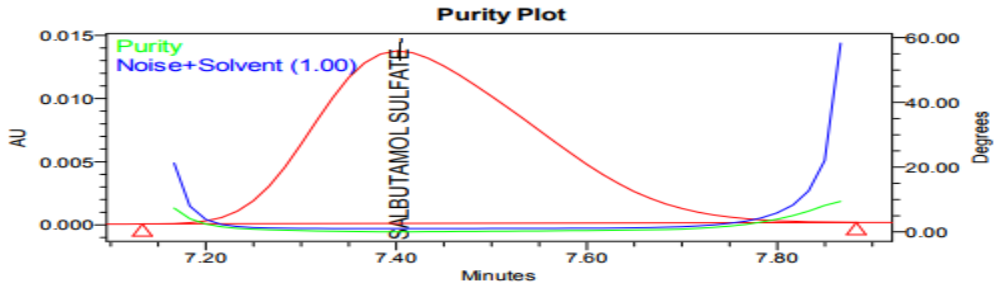
Name	RT	Area	% Area	RT Ratio
	4.006	1704	0.06	
	4.006	1735	0.06	
	5.686	65071	2.31	
	5.902	112096	3.97	
IMPURITY - J	6.695			
SALBUTAMOL SULFATE	7.207	2163733	76.66	
	9.686	159593	5.65	
	9.996	22082	0.78	
IMPURITY – C	11.516	35361	1.25	1.60
IMPURITY – N	11.949	4048	0.14	1.66
IMPURITY – D	12.314	133880	4.74	1.71
IMPURITY - F	12.744	2523	0.09	1.71
	12.998	15912	0.56	
IMPURITY - O	13.336	60918	2.16	1.85
	13.689	1630	0.06	
	14.095	8094	0.29	
	14.343	5447	0.19	
	14.574	4195	0.15	
	21.091	24569	0.87	

Figure-9: Chromatogram of Acid Degradation



Peak results

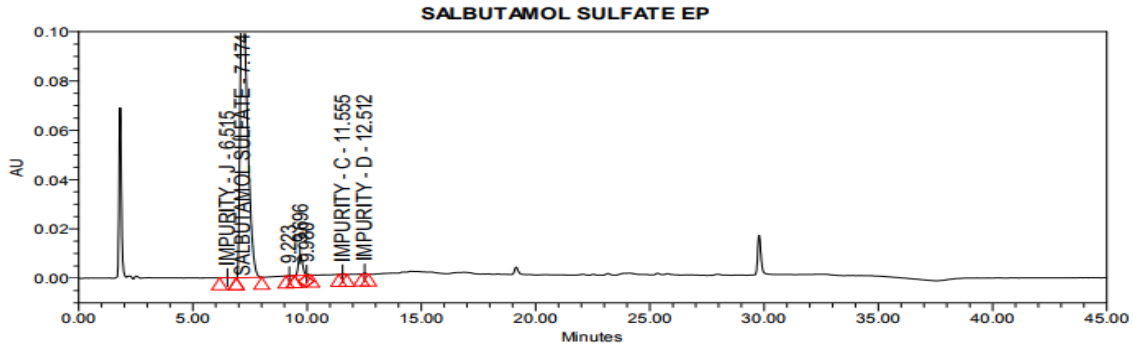
Name	RT	Area	% Area	RT Ratio
SALBUTAMOL SULFATE	7.404	216636	100.00	



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.230	1.090

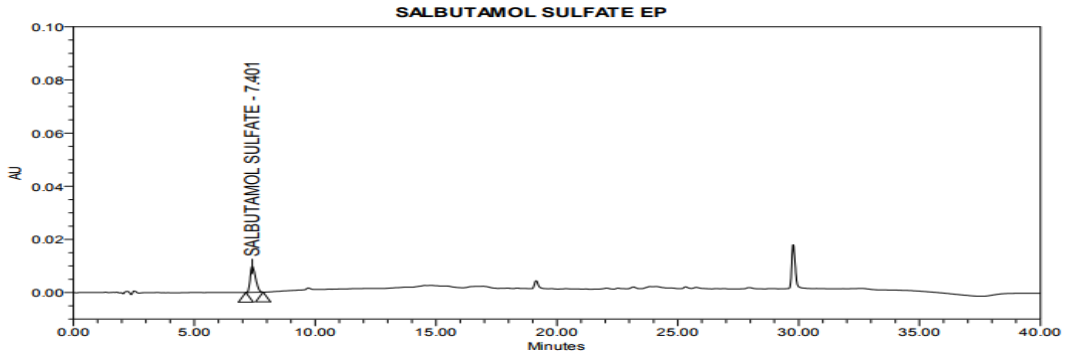
Figure-10: Chromatogram and peak purity of Acid Degradation (Diluted)



Peak Results

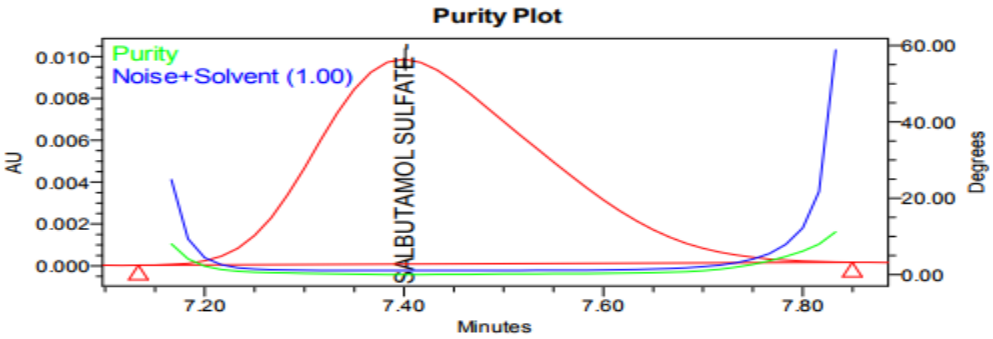
Name	RT	Area	% Area	RT Ratio
IMPURITY - J	6.515	1946	0.07	0.91
SALBUTAMOL SULFATE	7.174	2613173	95.53	
	9.223	1412	0.05	
	9.696	108015	3.95	
	9.980	3369	0.12	
IMPURITY – C	11.555	3570	0.13	1.61
IMPURITY - N	11.707			
IMPURITY - D	12.512	3851	0.14	1.74
IMPURITY - F	12.758			
IMPURITY - O	13.265			

Figure-11: Chromatogram of Alkali Degradation



Peak results

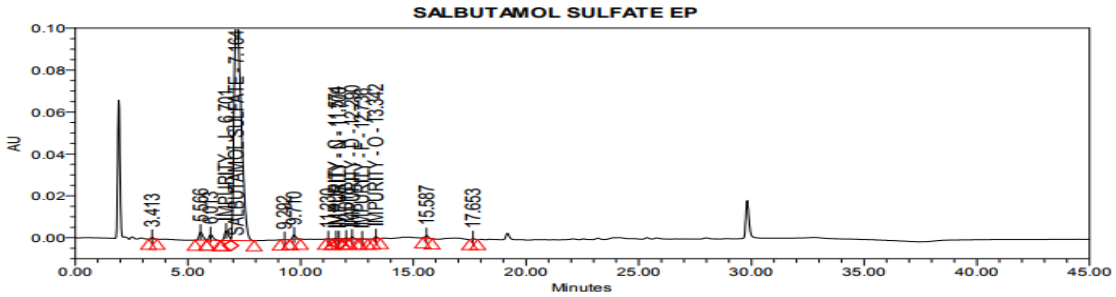
Name	RT	Area	% Area
SALBUTAMOL SULFATE	7.401	151199	100.00



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.202	1.129

Figure-12: Chromatogram and peak purity of Alkali Degradation (Diluted)

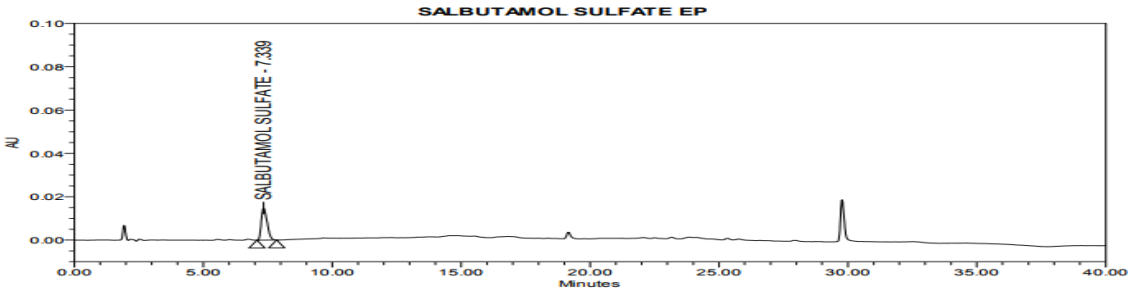


Peak Results

Name	RT	Area	% Area	RT Ratio
	3.413	7785	0.30	
	5.566	43989	1.72	
	6.013	32085	1.25	
IMPURITY - J	6.701	55143	2.15	0.94
SALBUTAMOL SULFATE	7.164	2354001	91.99	

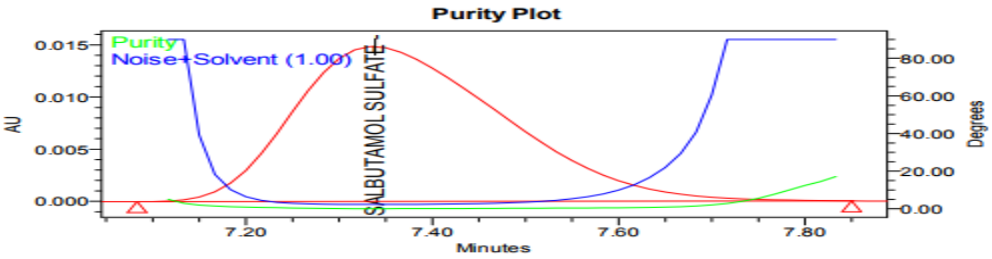
	9.292	1718	0.07	
	9.710	21161	0.83	
	11.230	1742	0.07	
IMPURITY – C	11.574	2959	0.12	1.62
IMPURITY - N	11.700	1064	0.04	1.63
	12.018	3681	0.14	
IMPURITY - D	12.290	8597	0.34	1.72
IMPURITY - F	12.736	1162	0.05	1.78
IMPURITY - O	13.342	8281	0.32	1.86
	15.587	11930	0.47	
	17.653	3547	0.14	

Figure-13: Chromatogram of Peroxide Degradation



Peak results

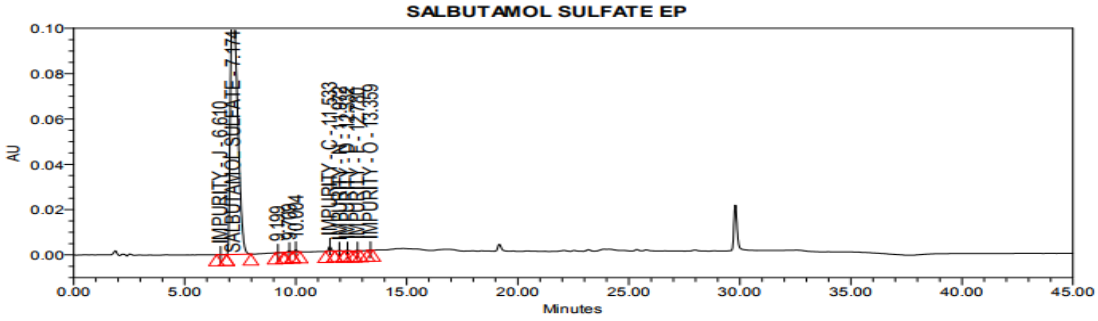
Name	RT	Area	% Area
SALBUTAMOL SULFATE	7.339	231341	100.00



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.271	3.138

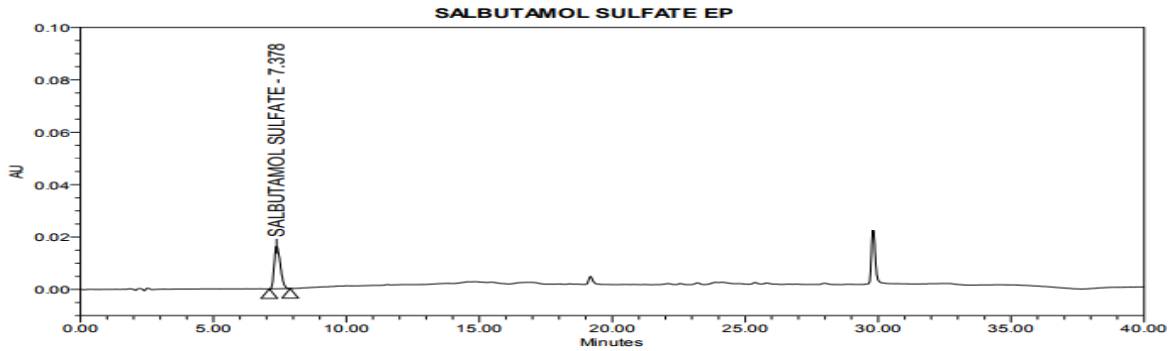
Figure-14: Chromatogram and peak purity of Peroxide Degradation (Diluted)



Peak Results

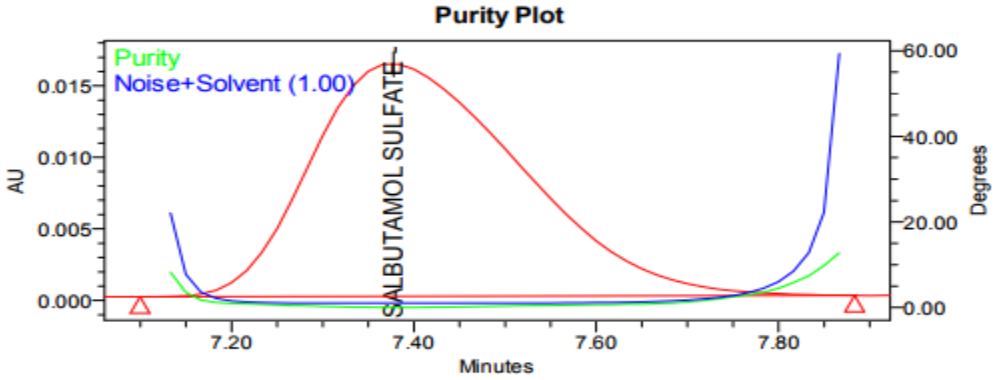
Name	RT	Area	% Area	RT Ratio
IMPURITY - J	6.610	1088	0.04	0.92
SALBUTAMOL SULFATE	7.174	2562401	98.42	
	9.199	1093	0.04	
	9.709	5802	0.22	
	10.004	7596	0.29	
IMPURITY – C	11.533	18014	0.69	1.61
IMPURITY - N	11.972	1772	0.07	1.67
IMPURITY - D	12.332	2380	0.09	1.72
IMPURITY - F	12.780	1765	0.07	1.78
IMPURITY - O	13.359	1553	0.06	1.86

Figure-15: Chromatogram of Reduction Degradation



Peak results

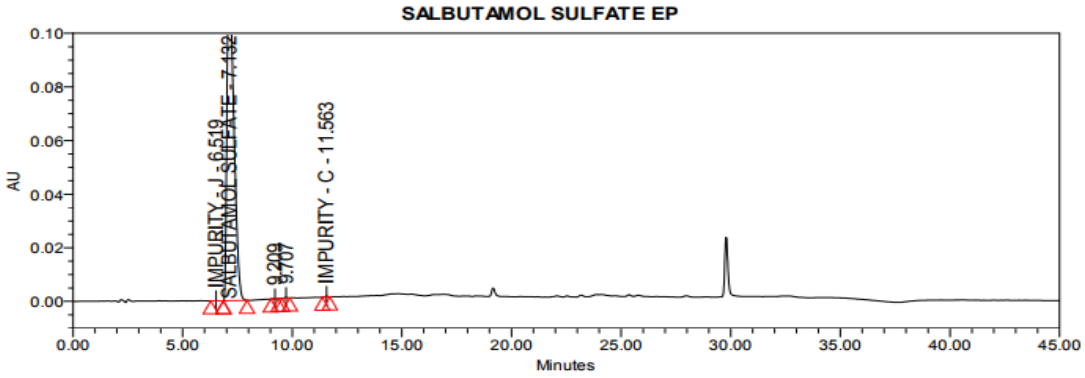
Name	RT	Area	% Area	RT Ratio
SALBUTAMOL SULFATE	7.738	259317	100.0	



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.272	1.065

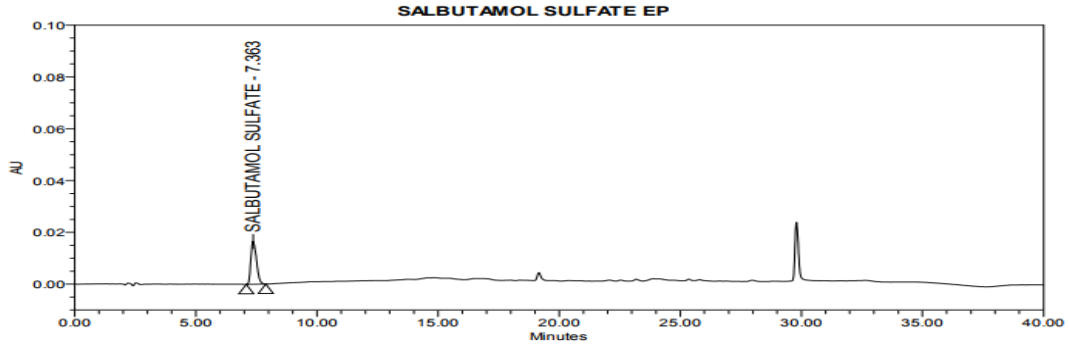
Figure-16: Chromatogram and peak purity of Reduction Degradation (Diluted)



Peak results

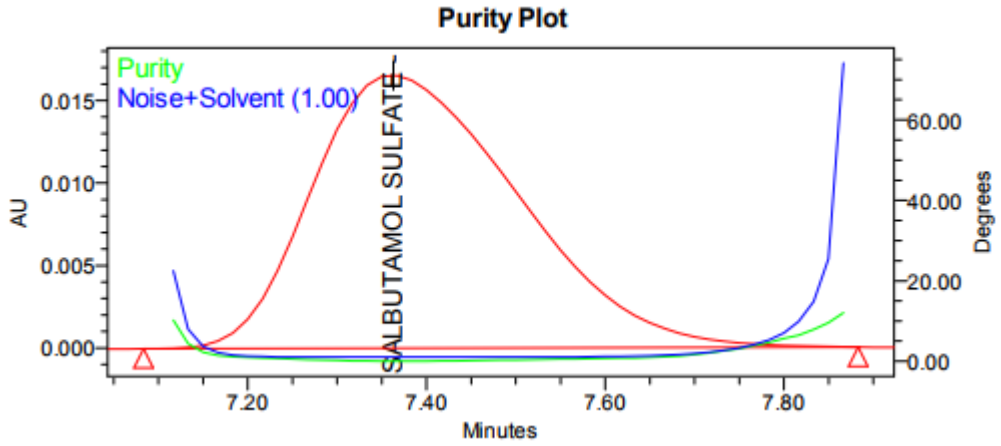
Name	RT	Area	% Area	RT Ratio
IMPURITY - J	6.519	1062	0.04	**0.91**
SALBUTAMOL SULFATE	7.132	2644465	99.70	
	9.209	889	0.03	
	9.707	2954	0.11	
IMPURITY - C	11.563	3090	0.12	1.92

Figure-17: Chromatogram of Hydrolysis Degradation



Peak results

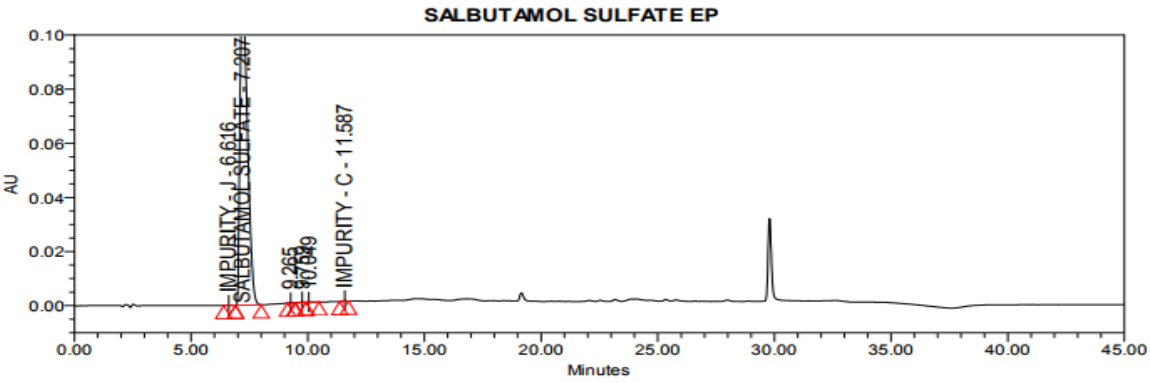
Name	RT	Area	% Area	RT Ratio
SALBUTAMOL SULFATE	7.363	264848	100.00	



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.289	1.064

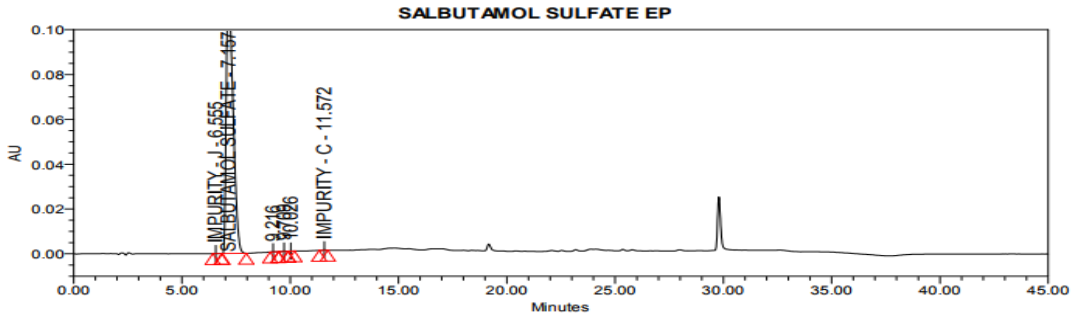
Figure-18: Chromatogram and peak purity of Hydrolysis Degradation (Diluted)



Peak Results

Name	RT	Area	% Area	RT Ratio
IMPURITY - J	6.616	823	0.03	**0.92**
SALBUTAMOL SULFATE	7.207	2600115	99.66	
	9.265	875	0.03	
	9.759	2336	0.09	
	10.049	1642	0.06	
IMPURITY - C	11.587	3304	0.13	1.61

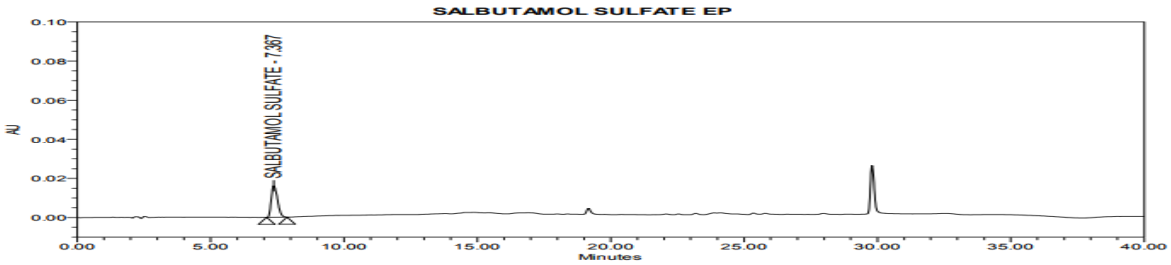
Figure-19: Chromatogram of Heat Degradation



Peak results

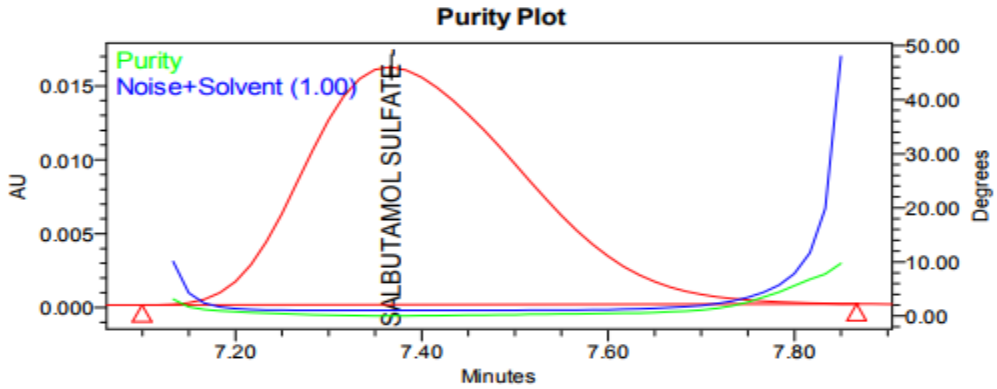
Name	RT	Area	% Area	RT Ratio
IMPURITY - J	6.555	740	0.03	0.92
SALBUTAMOL SULFATE	7.157	2572875	99.68	
	9.216	1300	0.05	
	9.709	2293	0.09	
	10.026	1019	0.04	
IMPURITY - C	11.572	2908	0.11	1.62

Figure-20: Chromatogram of Thermal Degradation



Peak results

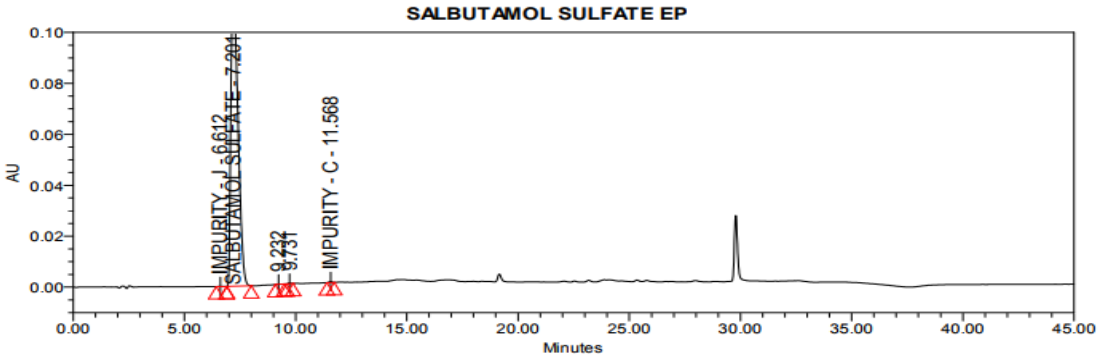
Name	RT	Area	% Area	RT Ratio
SALBUTAMOL SULFATE	7.367	257951	100.00	



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.209	1.066

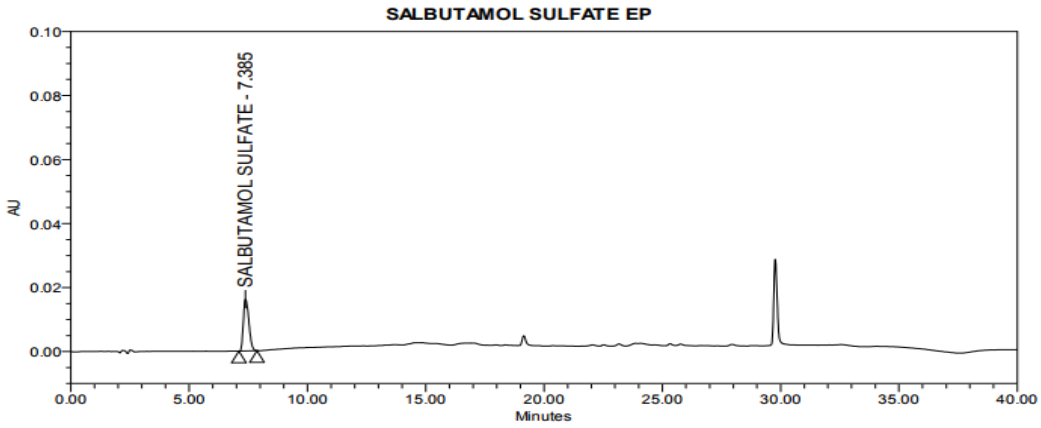
Figure-21: Chromatogram and peak purity of Thermal Degradation (Diluted)



Peak results

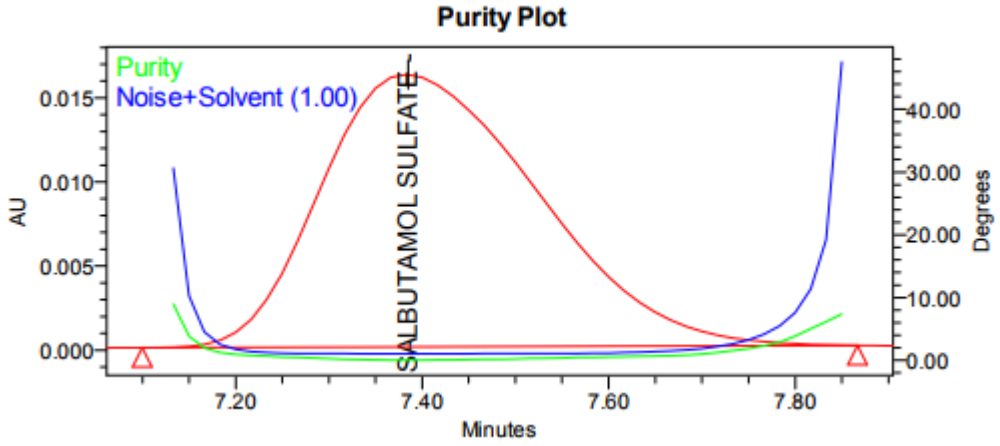
Name	RT	Area	% Area	RT Ratio
IMPURITY - J	6.612	956	0.04	0.92
SALBUTAMOL SULFATE	7.201	2622402	99.69	
	9.232	1426	0.05	
	9.731	2385	0.09	
IMPURITY - C	11.568	3341	0.13	1.61

Figure-22: Chromatogram of Humidity Degradation



Peak results

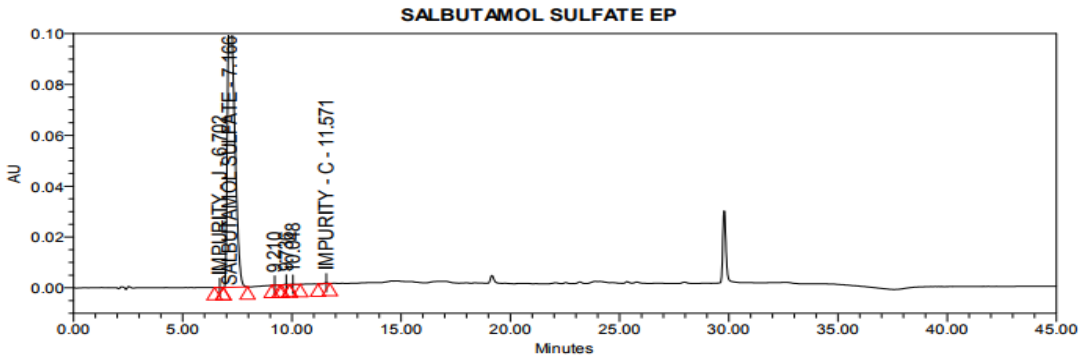
Name	RT	Area	% Area	RT Ratio
SALBUTAMOL SULFATE	7.385	261615	100.00	



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.231	1.079

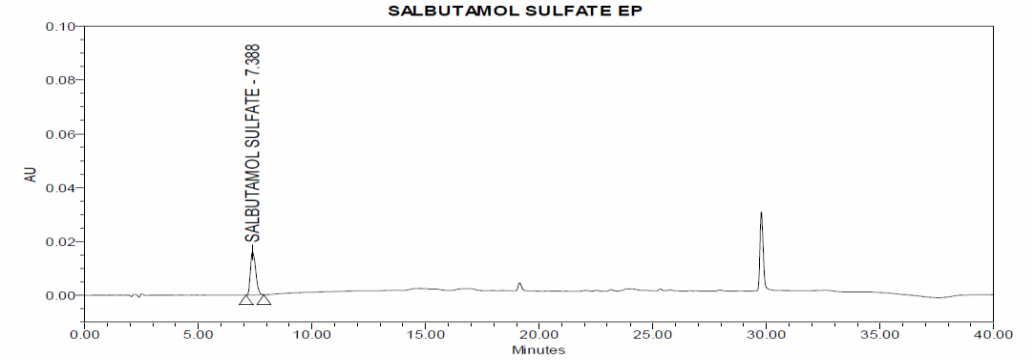
Figure-23: Chromatogram and peak purity of Humidity Degradation (Diluted)



Peak results

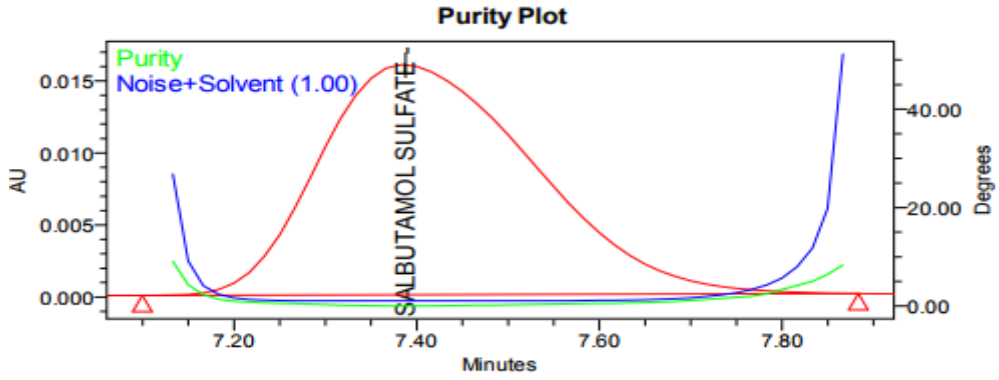
Name	RT	Area	% Area	RT Ratio
IMPURITY - J	6.702	551	0.02	0.94
SALBUTAMOL SULFATE	7.166	2592345	99.68	
	9.210	1066	0.04	
	9.736	2390	0.09	
	10.048	942	0.04	
IMPURITY - C	11.571	3407	0.13	1.61

Figure-24: Chromatogram of Photolytic Degradation



Peak results

Name	RT	Area	% Area	RT Ratio
SALBUTAMOL SULFATE	7.388	260614	100.00	



Peak results

SALBUTAMOL SULFATE	Purity angle	Threshold
	0.239	1.068

Figure-25: Chromatogram and peak purity of Photolytic Degradation (Diluted)

V. Conclusion

Six Impurities were identified BY HPLC method for determination of Related Substances of Salbutamol in Salbutamol API. is The Mean Recovery for known Impurities is within limits. Therefore, the HPLC Method for the determination of Related Substances of Salbutamol in Salbutamol API is accurate. The correlation coefficient for Salbutamol API, Impurities –J, C, N, D, F &O is more than 0.99. Therefore, the HPLC Method for the determination of related Substances of Salbutamol in Salbutamol 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. A stability indicating HPLC method for simultaneous estimation of Salbutamol in bulk and pharmaceutical dosage forms is established. The method is simple, accurate, linear, sensitive and reproducible as well as economical for effective quantitative analysis of Salbutamol in bulk and combined dosage forms. The method was validated; all method validation parameters were tested and shown to produce satisfactory results. The method is free from interactions of other ingredients and excipients used in formulations. Finally, concluded that method is suitable for use in routine quality control analysis of Salbutamol in active pharmaceutical ingredients and in pharmaceutical dosage forms.

Acknowledgments

The authors wish to thanks to the Director and HOD, Institute of Science, Mumbai for providing research facility. We also thanks to Satish Wagh, MD, Saloni Wagh, Shivani Wagh and Prashant Zate, Supriya Life Science Ltd, Mumbai for their cooperation and help to carry out this research work.

References

[1]. J.G.Baker, The selectivity of beta-adrenoceptor antagonists at the human beta1, beta-2 and beta-3 adrenoceptors. British Journal of Pharmacology, *144(3)*, 2005, 317-22.
[2]. L.K.Chong, K.Suvarna, R.Chess-Williams and P.T.Peachell, Desensitization of beta-2-adrenoceptor-mediated responses by short-acting beta2-adrenoceptor agonists in human lung mast cells. British Journal of Pharmacology, *138(3)*, 2003, 512-520.
[3]. N.G. Rathod and M.V. Lokhande, Characterisation and Identification of Process Related Impurity in Amodiaquine. HCl by Using Some Analytical Techniques: A Review, American Journal of Advanced Drug Delivery, *3(5)*, 2015, 264-284.
[4]. ICH Q2 (R1) Validation of Analytical Procedures: Definitions and Methodology, 2005.
[5]. C.P. Maurya and M. V. Lokhande, Impurity Profile and Validation of Pharmaceutical (API) Bulk Drug. World Journal of Pharmaceutical Research. *6(11)*, 2017, 878-887.
[6]. Guidelines on General Principles of Process Validation, CDER, US-FDA, 1987.
[7]. ICH, Q2 (A). Validation of analytical procedures: text and methodology International Conference on Harmonization. Geneva: 2005; 1-13.
[8]. Muhammad, T.K. Muhammad, U. Gul, M. K. Sattar, B. A. Hafsa, B and Aisha, S., HPLC Method Development and Validation for the estimation of Esomeprazole in Bulk and Pharmaceutical Dosage Form. International Journal of Drug Delivery and Research. *4(4)*, 2012, 252-256.
[9]. M.K.Gupta, M.V. Lokhande and N.G. Rathod, The antimalarial effect of Amodiaquine in humans. Life Sciences. Structural Elucidation of Process Related Bulk Drug By GCMS, NMR and IR techniques, International Journal of Medical Pharmaceutical Science,*s3*, 2013, 107-117
[10]. S.K. Churi and M.V. Lokhande, Identification and Impurity Profiling of Process Related Impurities In DTPEE, European Journal of Biomedical and Pharmaceutical Sciences, *4(9)*,2017, 617-623.

IOSR **Journal of Applied Chemistry** (IOSR-JAC) is UGC approved Journal with Sl. No. 4031, Journal no. 44190.

Dr. Manohar V. Lokhande, "Validation of Pharmaceutical (API) Bulk Drug by HPLC Methods. " IOSR Journal of Applied Chemistry (IOSR-JAC), vol. 11, no. 1, 2018, pp. 01-20.
Email: manohar2210@gmail.com