Synthesis, Isolation and Characterization of Vilanterol Bis Dichloro Benzene Ethane Impurity

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Abstract:

The objective of this study is to synthesize and isolate in process impurity of Vilanterol. In the process related impurity ranging from 0.05% to 0.2% in Vilanterol were detected by a gradient reversed phase high performance liquid chromatography (RP-HPLC). This impurity was isolated from the crude sample of Vilanterol using gradient reversed-phase preparative high performance liquid chromatography.

The unknown impurity inprocess impurity of Vilanterol was synthesised and isolated by using the preparative chromatography of purity above 95%. The pure impurity was characterized by using IR, NMR, and MS spectral data and confirm the molecular structure of IUPAC name 1,1'-[ethane-1,2-diylbis(oxymethylene)] bis (2,6-dichlorobenzene).

The Bis dichloro benzene ethane of vilanterol impurity was synthesized properly by using modern machineries. This is a work for the benefit of the human beings because impurity in the drugs can affect the human body.

Keywords: Vilanterol, Impurities, Spectroscopy, Identification, Characterization and synthesis.

Introduction:

Vilanterol is an anti-adrenoceptor agonist and is the commercially most widely used in clinical treatment of asthma and chronic obstructive pulmonary disease drugs. Currently commercially available anti- adrenergic agonists longest duration of action of 12 hours, for an operation has led to twice daily dosing[1, 2]. Over the last decade, the development of high potency, high selectivity, rapid onset, long duration of action, the once daily dosing – adrenoceptor agonist caused great concern in the pharmaceutical industry. Glaxo Group Limited developed a new type of ultra- long- acting age- adrenergic receptor agonist, on 18 December 2013 by the US FDA clearance to market its drug name as Anoro Ellipta [3, 4].

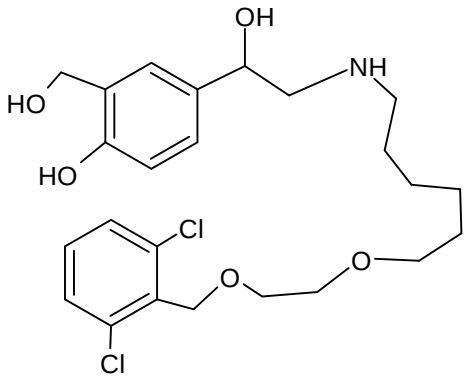


Figure No.1: Molecular structure of Vilanterol API

A literature search revealed that only analytical procedure is available for determination of Vilanterol from biological matrices, but nobody has reported synthesis, isolation, and characterization of impurity in the purified form, from Vilanterol API. The present communication involves the isolation, preparation of impurity and characterization by chromatographic and spectroscopic technique.

Materials and Methods

The raw material of Vilanterol impurity was received from Elitesynth laboratory Mumbai, India. The HPLC grade acetonitrile and methanol solvents were obtained from Merck co, Mumbai, India. The HPLC grade Formic acid was obtained from Sigma Aldrich, Mumbai, India. 2, 6 dichloro benzyl bromide, Caustic soda laboratory grade reagents were obtained from Sigma Aldrich, Mumbai.

An Agilent HPLC system equipped with 1100 series low pressure quaternary gradient pump along with pulse dampener, Photo diode array detector with auto liquid sampler handling system has been used for the analysis of the sample. An Inertsil ODS 3V, (4.6mm x 25cm x 5µ) column was employed for the testing of reaction mass of Vilanterol impurity. The column eluent was monitored at detection wavelength 225nm and 275nm. The mobile phase-A was 0.1% Ammonia make pH-7.5 using Formic acid and mobile phase-B was Acetonitrile. After the preparation of both the mobile phase degas and sonicate properly [5].

[**1]** 0.1% Ammonia: Acetonitrile gradient elution is as given in table

TIME(Mins)	Mobile Phase A	Mobile Phase B
0-5	70	30
5-20	10	90
20-24	10	90
24-24.1	70	30
24.1-30	70	30

Data was recorded by using Chem. station software.

Results and discussion

Preparative HPLC is the technique of choice for compound isolation and purification within the pharmaceutical and life science industries. Agilent technologies purification solution from nano gram to gram sample quantities. Agilent 1200 Series purification system with low delay volumes optimized for high recovery and purity, with PDA detector and flow rate is 0.001 to 100 ml/min with max. Pressure 400 bar. ODS-C18 250mm x 21.2mm x 10µ reverse phase silica column was employed for the separation of Vilanterol in process impurity. Solvent used for the separation was Water (0.1% formic acid): ACN with flow rate of 20 ml/min, with the detection of 225nm.and chromatography was performed at room temperature.

[2] Water (0.1% formic acid): ACN **gradient elution is as given in table**

TIME(Mins)	Mobile Phase A	Mobile Phase B
0-5	80	20
5-20	30	70
20-24	30	70
26-28	80	20
29-30	80	20

Mass Spectrometry (LC-MS/MS):

The LC–mass spectrometry (MS) and MS-MS studies were carried out on an Ion trap 6320 series electron spray ion trap spectrometer (Agilent Technologies). The source voltage was kept at 3.0 kV. Parameters: nebulizer gas = 30psi; dry gas = 3 L/min; dry temperature= 150 °C; capillary voltage =24500 to 21500 V. Nitrogen was used as both a sheath and auxiliary gas. Mass range was kept at *m/z* 50–600.The chromatography conditions and mobile phase are an Inertsil ODS 3V, (4.6mm x 25cm x 5µ) column was employed for the testing of reaction mass of Vilanterol impurity. The column eluent was monitored at detection wavelength 225nm and 275nm. The mobile phase-A was 0.1% Ammonia to adjust pH to 7.5 using formic acid and mobile phase-B was Acetonitrile. After the preparation of both the mobile phase degassing and sonication is done [6, 7].

[**3]** 0.1% Ammonia: Acetonitrile **gradient elution is as given in table**

TIME(Mins)	Mobile Phase A	Mobile Phase B
0-5	70	30
5-20	10	90
20-24	10	90
24-24.1	70	30
24.1-30	70	30

Nuclear Magnetic Resonance (NMR):

The 1H, and 13C nuclear magnetic resonance (NMR) spectroscopy experiment of the impurity was carried out at a frequency of 500 MHz at 25°C on an NMR spectrometer (Varian, Palo Alto, California). 1H chemical shifts are reported on the δ scale in ppm relative to tetra methyl silane 0.00 and $CDCl_3$ (δ=77.00ppm) and DMSO, D6 (δ=39.50ppm) respectively. ^{1}H experiments were run using a mixing time of 1000ns.

Fourier Transfer- Infra Red (FT-IR) Spectroscopy:

The IR spectra were recorded in the solid state as KBr dispersion medium using Perkin Elmer spectrum 100 FT-IR spectrophotometer.

Synthesis of impurity:

Charge ethylene glycol into RBF with mechanical stirrer. Add Potassium tert butyl oxide into it. After adding raw materials stir the reaction mass and add 2, 6 dichloride benzyl bromide into the reaction mass. After that heat the reaction mass to reflux with vigorous stirring. After 5 hrs of reflux reaction mass was checked in HPLC.

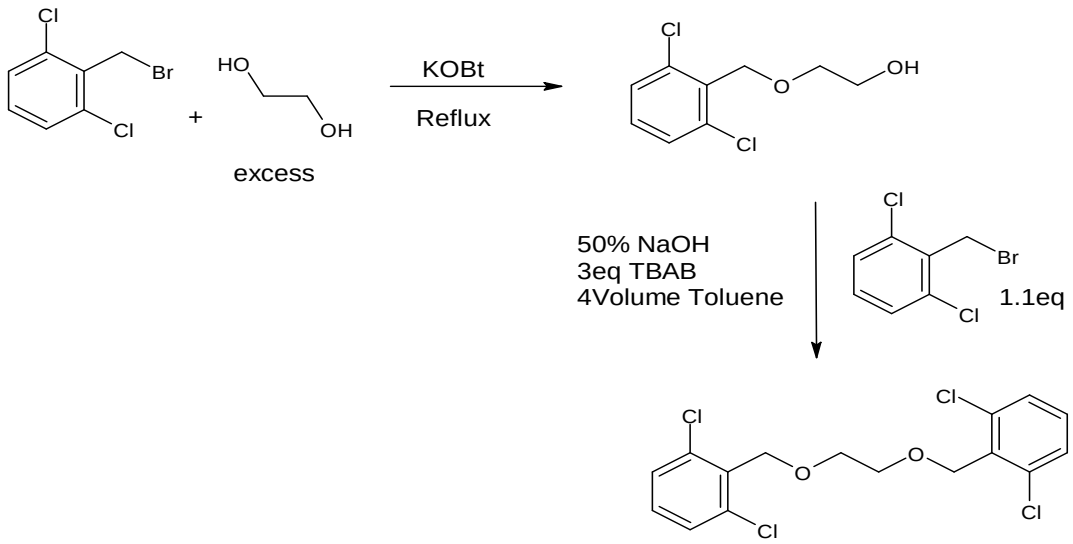


Figure No.3: Step-2 Reaction Scheme

Reaction mass was checked in HPLC and got the required impurity of 70% purity, reaction mass was then checked in LCMS for the confirmation of the required impurity mass. To improve the percentage of the required impurity for the characterization of the impurity preparative chromatography was used [8, 9].

Results and discussion:

Detection of impurity by HPLC:

Typical analytical HPLC chromatogram of the reaction mass of vilantrol impurity obtained by using the HPLC method discussed under the heading "High performance Liquid Chromatography (analytical)" [10]. The targeted impurity under study is marked observed at 24 min in the given HPLC chromatogram. It was given in figure No.4

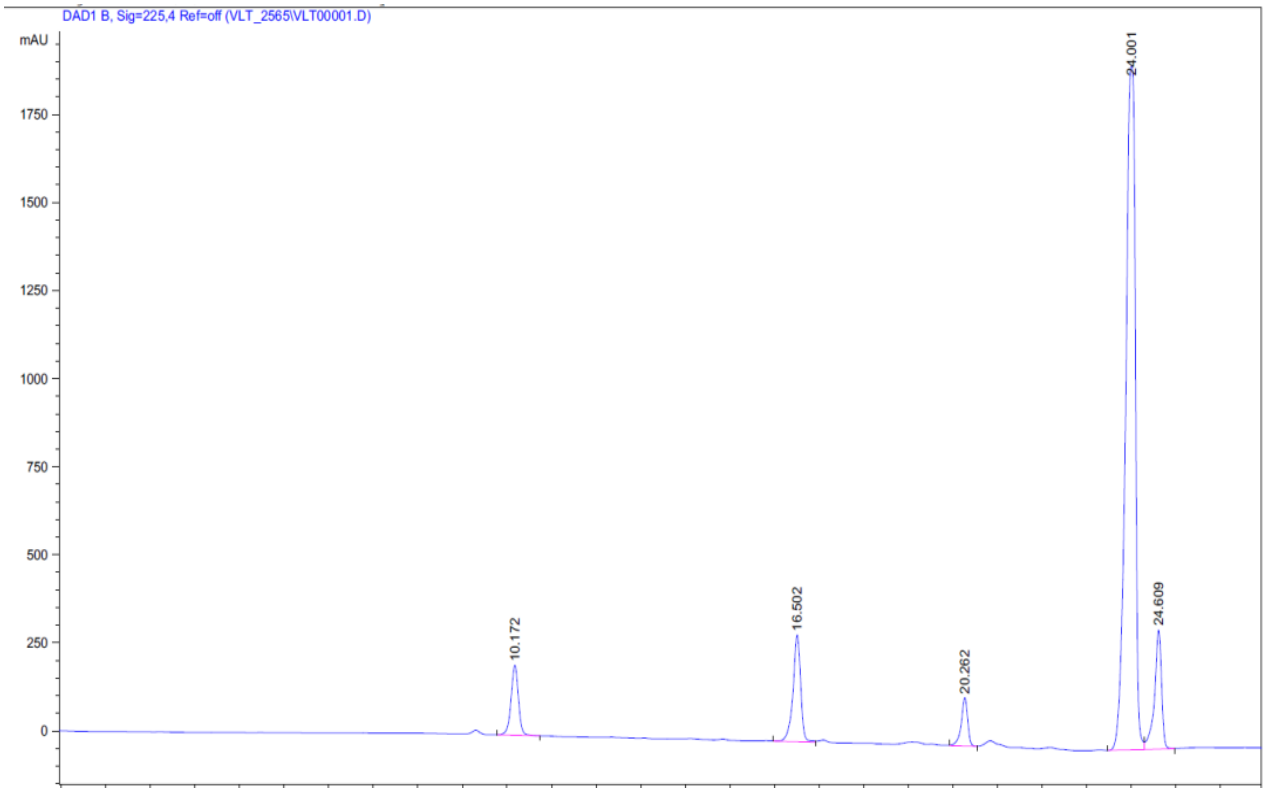


Figure No.4: HPLC Chromatogram of reaction mass

Isolation of the impurity by Prep-HPLC:

A simple reverse phase chromatographic system, discussed under the heading, "High performance Liquid Chromatography (preparative) was used for isolation of the impurity. After isolation of impurity, impurity fraction was concentrated at room temperature under high vacuum on a Buchii Rotavapour Model R124, the residue was then lyophilized for getting solid impurity. Purity of the impurity was tested in analytical method discussed under the heading, "High Performance Liquid Chromatography" (HPLC). The purity was found to be 95.17 % and it is exhibited in Figure No.5, before carrying out spectroscopic experiments.

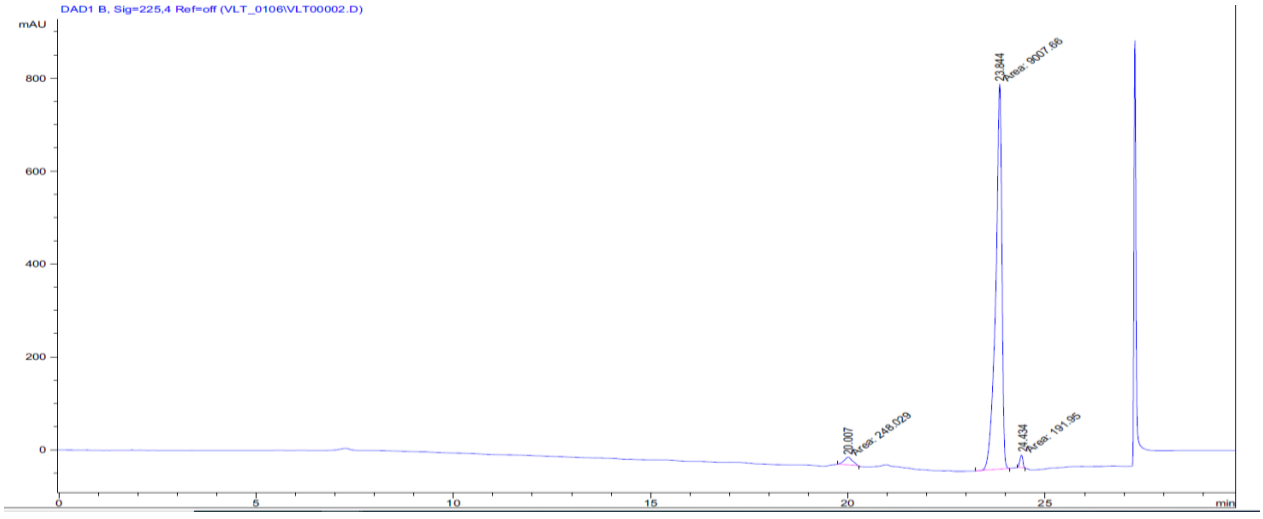


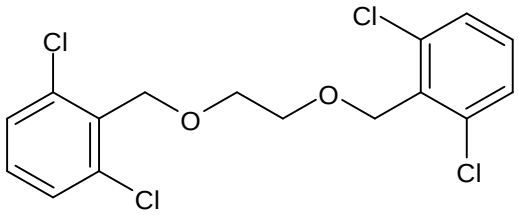
Figure No.5: HPLC Chromatogram of pure Impurity

LC-MS/MS Analysis:

LC-MS/MS analysis of Vilanterol impurity was performed using the chromatographic system as described under the heading "Mass Spectrometry (LC-MS/MS)". Result of LC-MS/MS analysis revealed that impurity exhibited molecular ion at m/z(M-1) = 397.88amu. it shows water adduct mass and it's MS/MS shown 359.8, 314.08, 242.12 amu.

Structure Elucidation:

The Molecular Structure of Vilanterol impurity is as Figure-6. The Molecular Formula is $C_{16}H_{14}Cl_4O_2$ and its molecular weight is 380.09amu and its monatomic mass is 380.09 amu.



1,1'-[ethane-1,2-diylbis(oxymethylene)]bis(2,6-dichlorobenzene)

Figure No.6: Molecular Structure of Vilanterol impurity

The IR spectra recorded in the solid state as KBr dispersion. FT-IR data of Vilanterol Impurity exhibited in the figure No.7. The mass spectra of Vilanterol impurity exhibited in the Figure No.8 and 9.

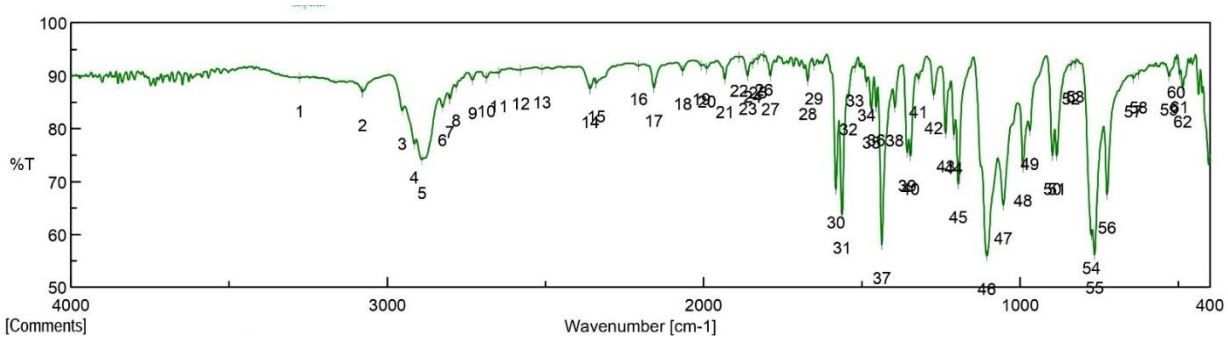


Figure No.7: IR spectra of Vilanterol Impurity

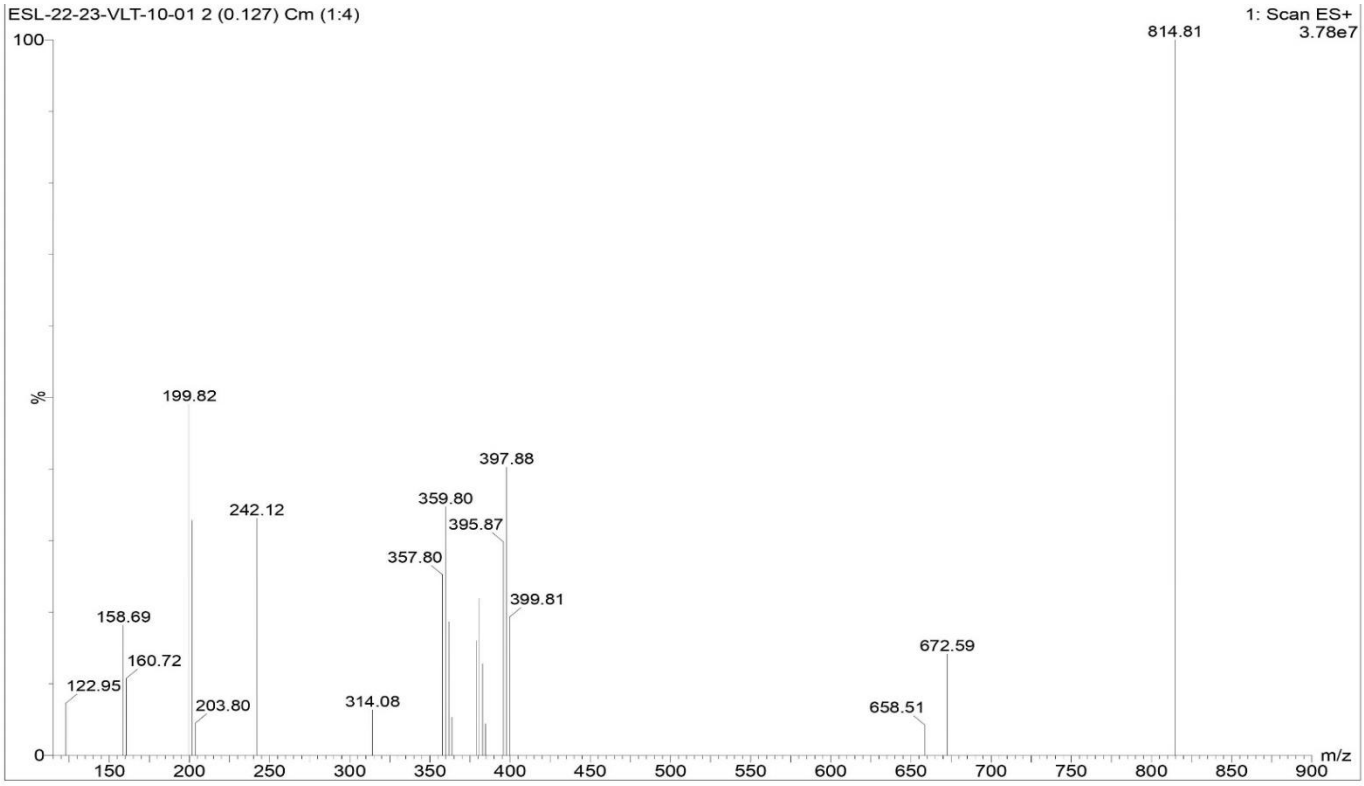


Figure No.8: MS/MS-MS spectra of Vilanterol Impurity

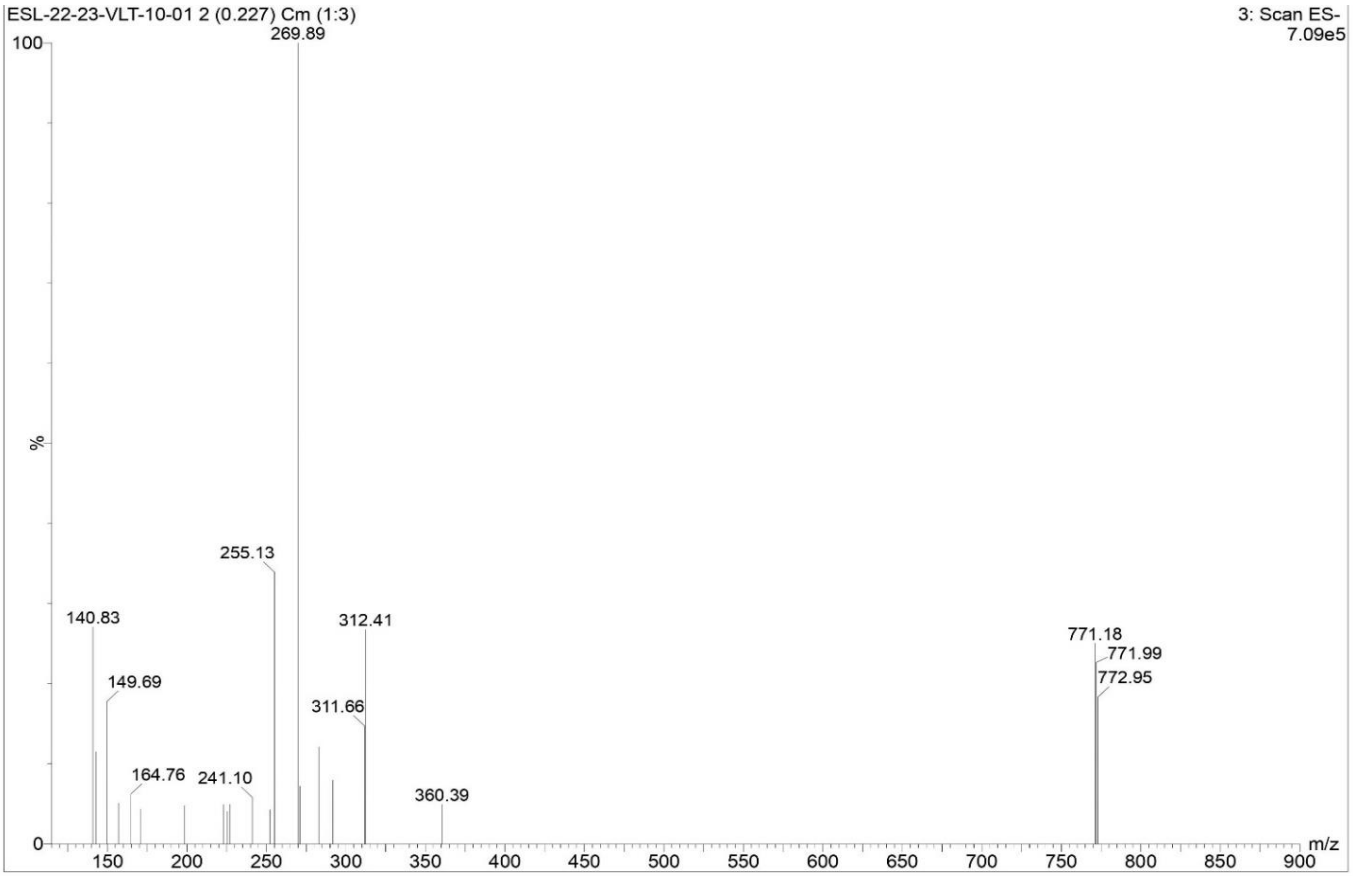


Figure No.9: MS/MS-MS spectra of Vilanterol Impurity

Sr. No.	Name of product	IR spectral data	MS, MS/MS data
1	Bis dichloro benzyl ethane Vilanterol impurity	C-O: 1150-1085. C-H: 2000-1650. C-Cl: 850-550.	m/z 397.88amu 359.8,314.08,242.12 amu

Table No.4: IR spectra and MS/MS-MS spectral data of Vilanterol Impurity

Vilanterol impurity1H NMR, and 13C NMR spectrum, for structure prediction and detailed assignment shown in table-2 and table-3. And was recorded in DMSO on 500MHz Varian instrument. 1H NMR and 13C NMR spectrum exhibited in the Figure No.10 and Figure No.11 respectively.

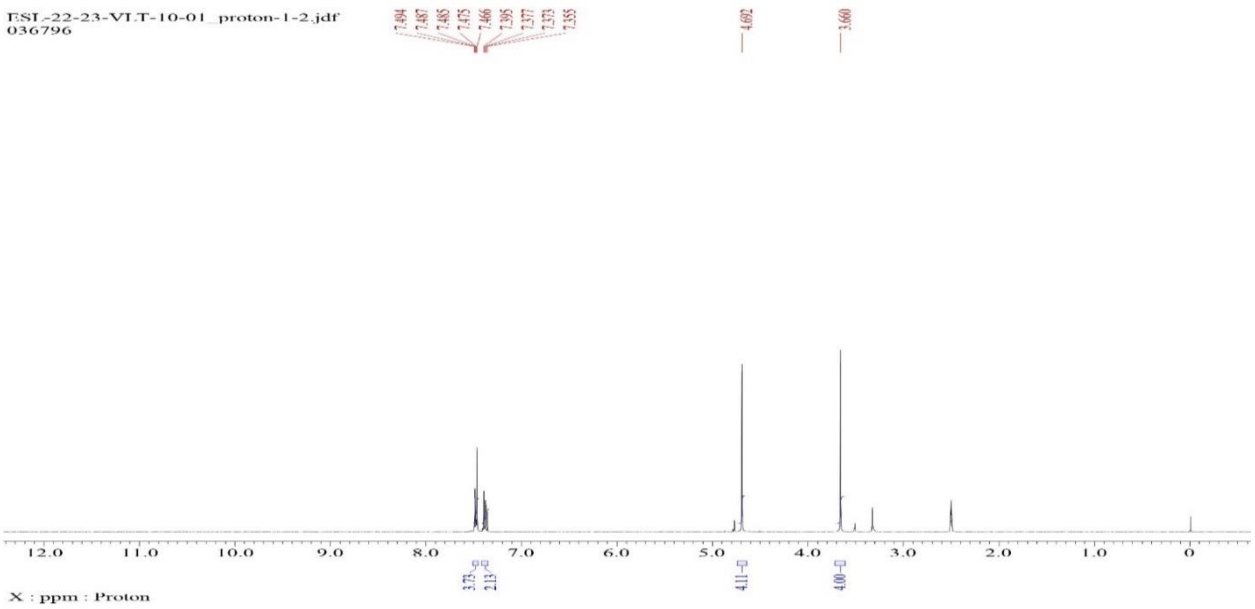


Figure No-10: 1H NMR spectrum of Vilanterol impurity

Table No: 5, 1H-NMR probable assignment of Vilanterol Impurity

Chemical Shift (ppm)	Number of Protons	Multiplicity	Assignment
7.494- 7.35	6	Multiplicity	Aromatic
4.692	4	Multiplicity	Aliphatic
3.66	4	Multiplicity	Aliphatic

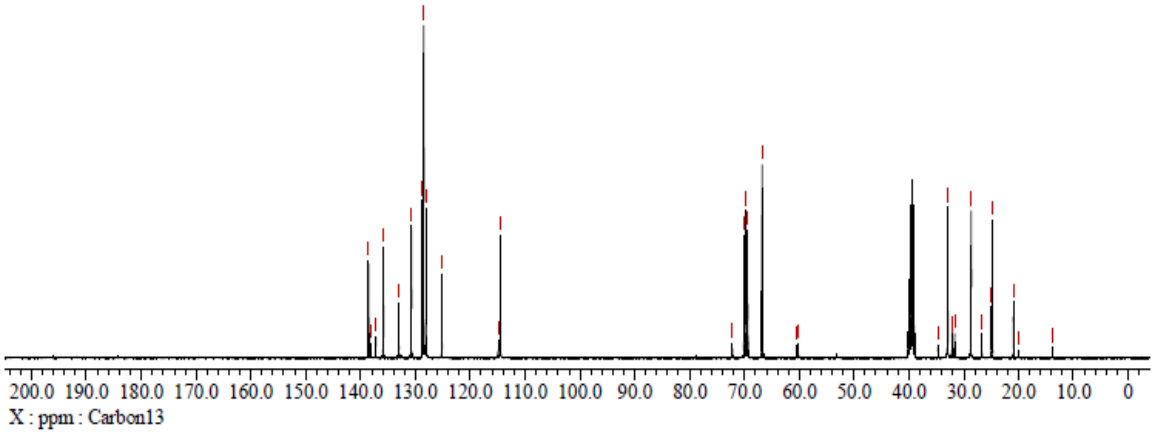


Figure No.11: 13C NMR spectrum of Vilanterol impurity

Table No:6, probable assignment for the Resonance Bands observed in the ^{13}C NMR spectrum of Vilanterol impurity

Chemical Shift (ppm)	Type of Carbon	Assignment
138.753	Aromatic	1
138.552	Aromatic	1
138.149	Aromatic	15
137.234	Aromatic	15
135.871	Aromatic	2
133.114	Aromatic	2
132.948	Aromatic	4
130.742	Aromatic	4
128.789	Aromatic	14
128.483	Aromatic	16
128.08	Aromatic	16
125.198	Aromatic	18
114.904	Aliphatic	18
114.55	Aliphatic	18
114.456	Aliphatic	11
72.199	Aliphatic	11

Conclusion:

This research paper describes the synthesis, isolation and structure elucidation of process related impurity of Vilanterol. The impurity was separated by reverse phase chromatographic technique, by using High performance liquid chromatography (prep-HPLC). The isolated impurity was characterized by using IR, 1H NMR, 13C NMR and LC-MS/MS spectroscopic technique. The synthesis of impurity was also discussed in brief.

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