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Isolation and characterization of forced degradative products of an anti psychotic drug levosulpiride by spectroscopic techniques

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ABSTRACT

A High Performance Liquid Chromatography (HPLC) method was developed for the determination of degradation products of an anti psychotic drug Levosulpiride. The chromatographic separation was achieved on Shimadzu LC-2010 with PDA system and Hypersil BDS C_{18} (250x4.6 mm) column using isocratic elution of mobile phase. The present study is aimed to degrade the drug using different forced degradation conditions (as per ICH guidelines) like acid, base, oxidative, thermal and light. One major degradation product was observed in the base and oxidative degradations while no degradation products were observed in acid, thermal and photolytic degradations respectively. The major degraded products were isolated using semi-preparative HPLC. The degraded products were subjected to LC-MS to find out the products mass. The structures of the degradative products were characterized using IR and NMR techniques.

Keywords: Levosulpiride, reverse phase HPLC

INTRODUCTION

Levosulpiride is substituted benzamide and is a anti psychotic drug, reported to have selective antagonist of dopamine D2 receptor activity on both central and peripheral Levels. It is commonly prescribed to patients with psychosis, depression and functional dyspepsia. At low doses, Levosulpiride increases dopaminergic neurotransmission, primarily by blocking of the dopamine auto receptors, which inhibits the presynaptic dopamine synthesis and release of dopamine. Chemically, Levosulpiride is [(S)-(-)-(amino sulfonyl)-N-[(1)-ethyl-2-pyrrolinyl) methyl]-2-methoxy benzamide], a antipsychotic agent belonging to the substituted benzamide group .Levosulpiride is a white solid powder; with melting point 183-186°C and molecular weight is 341.43.

Levosulpiride is not listed officially in any pharmacopeia and is listed in the Merk index [1] and Martindale [2]. It has D2-dopamine receptor antagonistic activity can cause anti depressive, antiulcer effects [3]. Comparison of racemic and dexto forms, the levo-form of sulpiride has maximum central anti dopaminergic activity [4], antiemetic, anti dyspeptic effects and lower toxicity [4]. Literature survey revealed that simple UV-Spectrometry [5], visiblespectrophotometry [6], spectroflurometry [7] and RP-HPLC [8] method for determination of Levosulpiride in bulk drug and formulation. Different studies [9, 10, 11, 12 and 13] were carried out on the drug molecule Levosulpiride has been in the market for more than 15 years, and demonstrated high efficacy in the control of dyspeptic symptoms and its favourable safety profile. A review [14] on the clinical pharmacology, therapeutic efficacy and tolerabity of Levosulpiride indicated that the incidence of adverse events were 11% in 840 patients with dyspeptic and most of them were mild. Levosulpiride at low doses increases dopaminergic neurotransmission, primarily by blocking of the dopamine auto receptors, which inhibits the pre synaptic dopamine synthesis and release of dopamine. Several Researchers [15, 16, 17, and 18] reported a simple and validated RP HPLC method for the estimation of Rabeprazole, Pantoprazole and Levosulpiride in bulk

drug pharmaceutical dosage forms. Literature survey [19] revealed about the development and validation of stability indicating RP-HPLC method for estimation of Levosulpiride and Rabeprazole sodium. Literature is not found on isolation and characterization of degradation products formed in forced degradation condition, which is very important information for the drug.

The present study is focussed on the improvement of the sensitivity and the selectivity of the chromatographic determination of Levosulpiride, a simple and reversed-phase HPLC method with UV detection at 282 nm, where all impurities have been separated in a single analytical column. Shimadzu HPLC has been successfully employed for the determination of Levosulpiride and its degraded products.

EXPERIMENTAL SECTION

Levosulpiride were obtained from SDS Labs PvtLtd, Navi Mumbai, India. Chemicals used in the present study include ammonium acetate, glacial aceticacid, sodium hydroxide, hydrochloric acid, hydrogen peroxide of analytical grade and HPLC grade solvents, acetonitrile were procured from Across or Rankem or S.D.Fine Chemicals Limited and Milli -Q plus purified water was used. A shimadzu LC 2010 separation module equipped with a photo diode array UV detector consists of column heater compartment. This system was controlled by LC –Solutions software. A validated reverse phase HPLC method was developed for the separation and quantification of Levosulpiride and its degraded products was developed using Hypersil BDS C_{18}(250x4.6mm) as a stationary phase. The wavelength at 282 nm was selected. Ammonium acetate buffer was prepared by adding 3.85 gm of ammonium acetate in 1000 ml water and p^H adjusted to 3.5. A mobile phase was used with the mixture of buffer and acetonitrile in the ratio 84:16.

Development of a chromatographic method by HPLC

The author developed a reverse phase HPLC method for the eluting the main drug Levosulpiride and the degradation products generated during the forced degradation studies. The conditions of the developed method were checked with reference to the pure drug.

The following forced degradation conditions were carried out as stipulated in ICH Q1A (R2) and Q1B guidelines [20-22].

Acid Hydrolysis: Levosulpiride (75 mg) was dissolved in 10 ml of 1N hydrochloric acid and kept at 25°C.The solution was monitored every hour for 48 h for the degradation products.

Base Hydrolysis: Levosulpiride (75 mg) was dissolved in 10 ml of 1N sodium Hydroxide and kept at 25°C.The solution was monitored every hour for 48 h for the degradation products

Oxidative degradation: Levosulpiride (75 mg) was dissolved in 10 ml of 5%w/v hydrogen peroxide solution and the solution was maintained at 25°C.The solution was monitored every hour for 48 h for the degradation products.

Thermal degradation: Levosulpiride solid was subjected to dryness and heated to 60°C for 10 days. This substance was dissolved in the diluent and this solution was analysed for degradation products. Every day required amount of sample was taken from the oven and the same procedure was adopted for monitoring the degradation products.

Photolytic degradation: Levosulpiride solid was exposed to UV and Fluorescent light for 1.2 million lux hours using cool white fluorescent lamp and 200 Watt h/m2 UV energy as specified in ICH Q1B guidelines21. After exposure to light for12 h in a day, the drug was dissolved in diluent and this solution was analysed for degradation products.

RESULTS AND DISCUSSION

Levosulpiride was subjected to different forced degradation conditions. In the present study HPLC method was developed for the separation of Levosulpiride and its degradation products. Degraded solutions of the drug were analysed in HPLC using the chromatographic conditions for identifying and characterizing the major degradation products. They were isolated using semi-preparative liquid chromatography and were characterized by different spectral techniques. The compound was degraded in basic and oxidative conditions and was stable in acidic, thermal and sunlight.

Analysis of base degradation sample by HPLC: The base hydrolyzed solution of Levosulpiride was diluted to the required concentration with diluents and analyzed using HPLC.The corresponding chromatogram depicts that Levosulpiride degraded significantly in basic conditions. The product of base degradation is at 3.667 mins and RRT value is 0.84 and 3.6% by area percentage. It is a new degradation product of base hydrolysis and is termed as BDP.

Analysis of Oxidative degradation sample by HPLC: The Oxidized solution of Levosulpiride was diluted to the required concentration with diluents and analyzed using HPLC. The corresponding chromatogram depicts that Levosulpiride degraded significantly in oxidative conditions. The product of oxidative degradation is at 5.367 mins and RRT value is 1.23 and 4.1% by area percentage. This newly formed compound is the degradation product of oxidation and is represented as ODP. The respective chromatograms of Levosulpiride (standard), base degradation and oxidative degradation products are presented in Figures from-1 to 3.

Structural elucidation of BDP& ODP: (a) LC-MS analysis was carried out for obtaining structural insight of the drug Levosulpiride, base degradation product and the oxidative degradation product. The mass spectra extracted a protonated molecular ion of Levosulpiride m/z at 342; base degradation product m/z at 214 and oxidative degradation product m/z at 358. The Mass spectra of Levosulpiride (standard), base degradation and oxidative degradation products are presented in Figure-4 to 6.

The data related to forced degradation from HPLC is presented in Table-1; The structural confirmation data is presented in Table-2.

^{1}H NMR & ^{13}C NMR spectral data of BDP and ODP are presented in Tables-3 to 5.

^{1}H NMR & ^{13}C NMR spectra of Levosulpiride, BDP and ODP are presented in Figures from 7 to12.

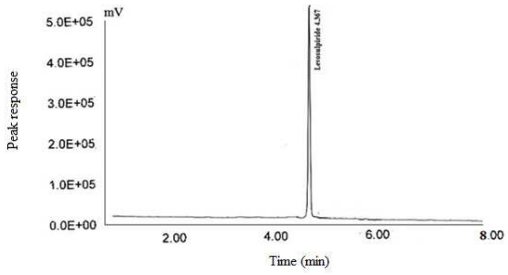


Figure-1: HPLC Chromatogram of Levosulpiride drug substance

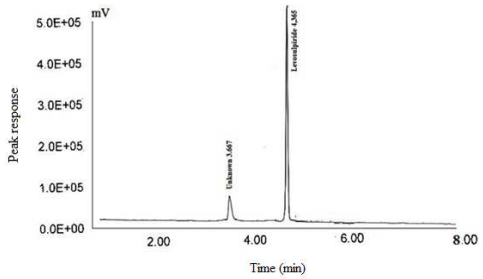


Figure-2: HPLC chromatogram of Base degradation

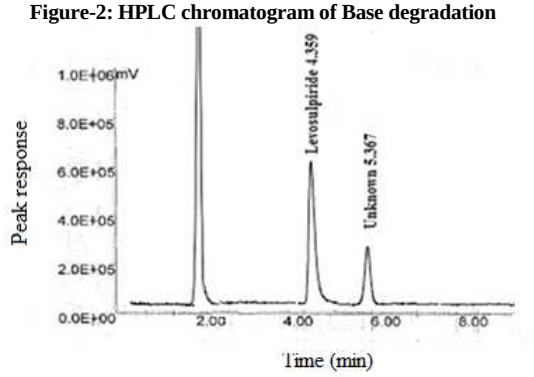


Figure- 3: HPLC chromatogram of Oxidative degradation

Table-1: Forced degradation data from HPLC

Name of the drug	Retention RT(min)	Relative Retention time RRT	Remarks
Levosulpiride	4.367	1.0	Drug substance
Acid degradation	4.371	0.99	No major degradation Product was identified
Base degradation	3.667	0.84	One major degradation Product was Identified
Oxidative degradation	5.367	1.23	One major degradation Product was Identified
Thermal (60°C) Treatment	4.368	0.99	No major degradation Product was Identified
Photolytic Treatment	4.369	0.99	No major degradation Product was Identified

The structural confirmational data of drug and degradation products is summarized in Table-2**.**

Table-2: Structural Conformational Data of drug and degradation products

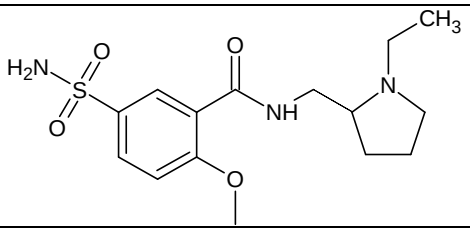
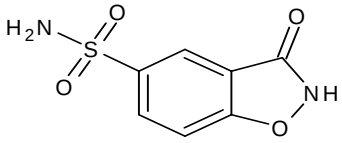
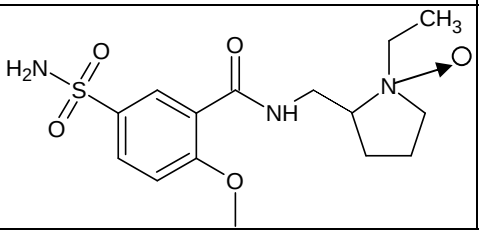
Compound	Structure	Molecular formula	Molecular Weight
Levosulpiride		$C_{15}H_{23}N_3O_4S$	342
Base degradation Product		$C_7H_6N_2O_4S$	214
Oxidative degradation Product		$C_{15}H_{24}N_3O_5S$	358

Table-3: Assignments of ^{1}H NMR and ^{13}C NMR Chemical shifts of Levosulpiride

Levosulpiride		
Position[1]	**^{1}H Chemical shift(ppm)**	**^{13}C Chemical shift(ppm)**
1	7.0(s,2H)-NH_2	-
2	-	130.5
3	7.99(d,1H)-CH	130.4
4	7.58(d,1H)-CH	111.8
5	-	158.9
6	-	135.8
7	8.64(s,1H)-CH	122.2
8	3.98(s,3H)-O-CH_3	61.3
9	C=O	163.2
10	8.29(s,1H)-NH	-
11	3.22(m,1H)-CH	52.6
12	3.65(m,1H)-CH	46.8
13	1.63(m,2H)-CH_2	27.3
14	1.85(m,1H)-CH	21.3
15	2.84(m,1H)-CH	-
16	3.22(m,4H)-2CH_2	40.4
17	2.21(m,2H)-CH_2	35.4
18	1.16(t,3H)-CH_3	13.4

1: (s) singlet,(d) doublet (t) triplet, (q) quartet, (m) multiplet, (dd) doublet of doublet

Table-4: ^{1}H NMR and ^{13}C NMR chemical shifts of BDP

Base Degradation product (BDP)	
^{1}H Chemical shift	**^{13}C Chemical shift**
7.00 (s,2H)-NH_2	-
-	130.4
8.01(d,1H)-CH	130.3
7.10 (d,1H)-CH	111.6
-	158.8
-	135.6
8.68(s,1H)-CH	122
-	
C=O	162.8
8.40(s,1H)-NH	-

Table-5: ^{1}H NMR and ^{13}C NMR chemical shifts of ODP

Oxidative Degradation product(ODP)	
^{1}H Chemical shift	**13C Chemical shift**
6.95(s,2H)-NH_2	-
-	130.4
7.98(d,1H)-CH	130.3
7.51(d,1H)-CH	111.6
-	158.9
-	135.7
8.62(s,1H)-CH	122.2
3.98(s,3H)-OCH_3	61.3
C=O	163.1
8.28(s,1H)-NH	-
3.20(m,2H)-CH_2	52.6
3.59(m,1H)-CH	45.9
1.59(m,2H),-CH_2	26.9
1.79(m,1H)-CH	20.1
2.79(m,1H)-CH	-
3.17m,2H)-CH_2	39.8
2.21(m,1H)-CH_2	35.3
1.15(t,3H)-CH_2CH_3	13.1

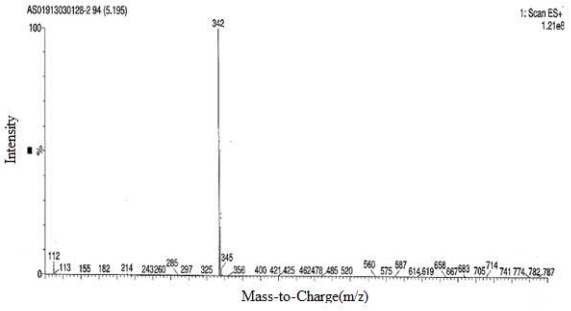


Figure-4: **Mass spectrum of the Levosulpiride Drug Substance**

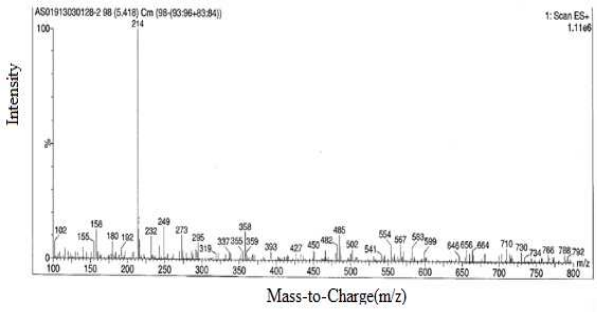


Figure-5: Mass spectrum of the base degradation product (BDP)

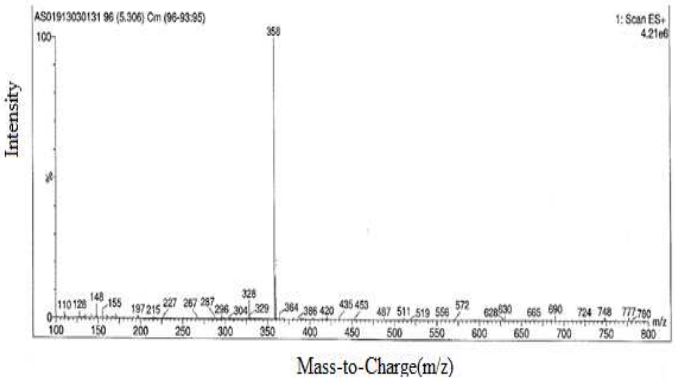


Figure-6: Mass spectrum of the oxidative degradation product (ODP)

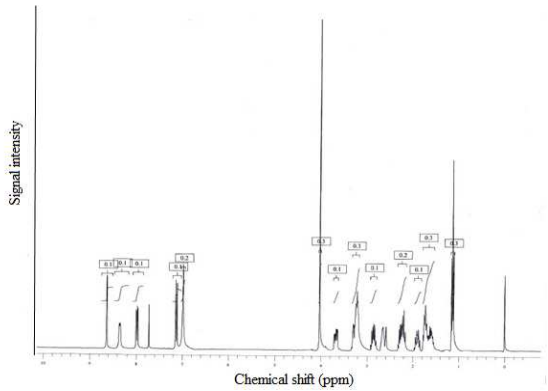


Figure-7: ^{1}H NMR spectrum of Levosulpiride

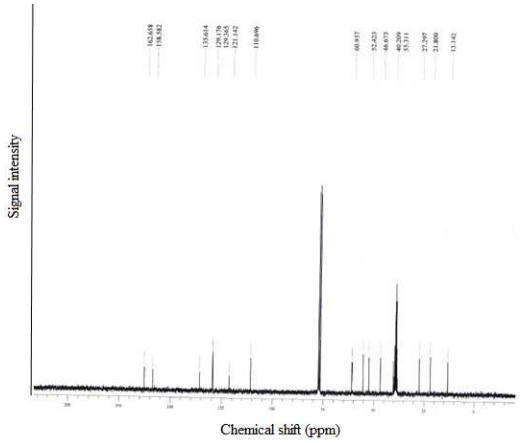


Figure-8: ^{13}C NMR spectrum of Levosulpiride

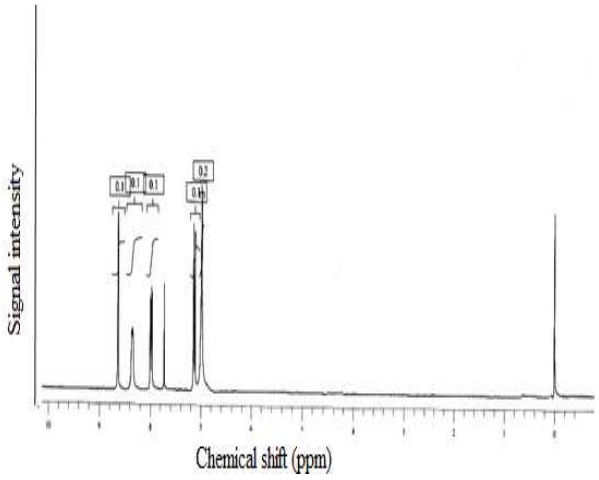


Figure-9: ^{1}H NMR spectrum of BDP

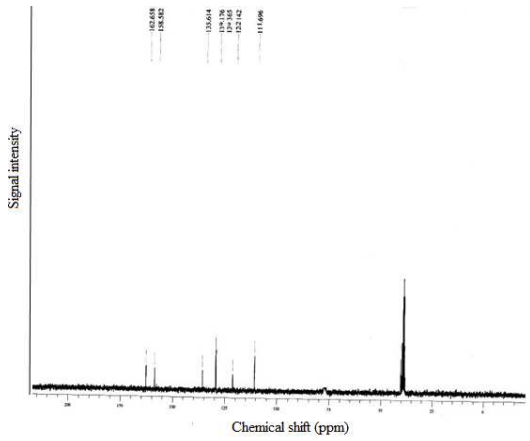


Figure-10: ^{13}C NMR spectrum of BDP

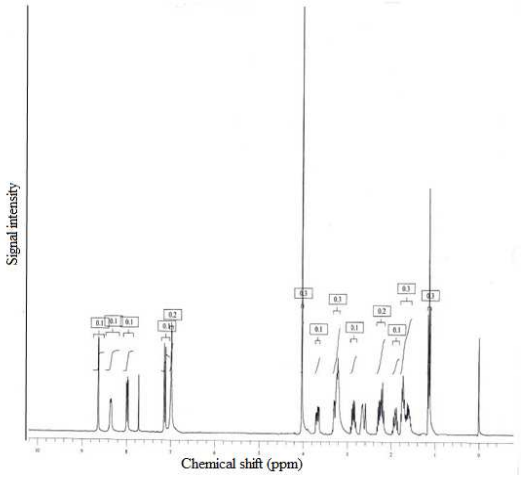


Figure-11: ^{1}H NMR spectrum of ODP

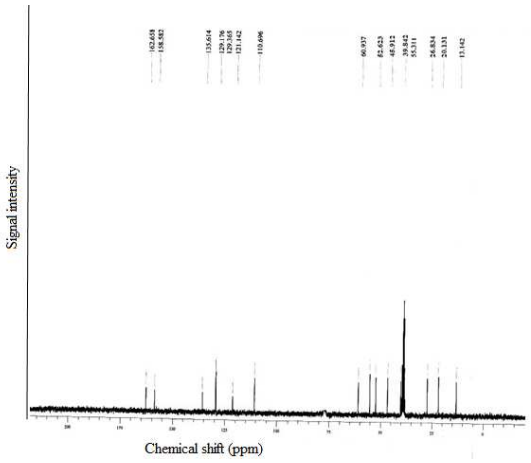


Figure-12^{13} C NMR spectrum of ODP

CONCLUSION

The present study revealed that the Levosulpiride drug molecule can produce the degradative products during base degradation and oxidative degradation while such degradative products were not identified by acid, thermal and photolytic degradation. The degradative products identified in the forced degradation by the semi-preparative HPLC and spectral studies which includes ^{1}H NMR, ,^{13}C NMR, Mass and IR confirmed the products as 3-oxo-2,3dihydro-

1,2-benzoxazole-5-sulfonamide by base degradation and S)-(-)-(amino sulfonyl)-N-[(1)-ethyl-2-pyrrolinyl) methyl]-2-methoxy benzamide –N-oxide by oxidative degradation.

The present research study is of its first type to identify the degradative product of Levosulpiride drug molecule. The present study on the base degradative and oxidative degradative products, confirm with a significant quantity. Hence the present study will become a bench mark in the base as well as the oxidative degradative studies of the Levosulpiride.

The present work also demonstrated the practical utility of NMR, LC-MS in the structural elucidation of the degradative products of Levosulpiride. It further suggested that present instrumental techniques are helpful to the researchers in characterizing the isolated degradation products in smaller quantities to carry out the structural elucidation in an effective way.

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