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Validated Stability Indicating HPLC Method for Identification of Degradant of Opipramol by LC-MS

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Abstract:

The objective of this method was to develop a sensitive, accurate and precise HPLC-PDA detector method for the estimation of opipramol in bulk drug & pharmaceutical solid dosage form. The stability indicating method for opipramol was validated in accordance with ICH guidelines and degradant characterization was carried out using LC-MS. Degradant products were separated by developed gradient LC method using Qualisil 5 BDS-C18 column 250 length, 4 mm diameter,5 µm particle size, with mobile phase of 0.02% of Orthophosphoric acid whose pH was adjusted to 3.26 (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) (60-40% V/V) were used. The flow rate was 1.0 ml/min, retention time was 4.91 min and effluents were monitored at 254 nm. LC-MS system controlled by HighStar 3.2 software and Bruker IMPACT-HD model equipped with ESI ionization source were used. The model had the mass resolution up to 50,000 FSR and mass detectable range from 100 to 3500 m/z. The proposed method was linear in concentration range of 10-70 µg/ ml with correlation coefficient of 0.999, mean % recovery 99.86 and precise. The drug was subjected to hydrolytic, oxidative, photolytic and thermal condition where it showed instability in hydrolytic (acidic ad alkaline) condition, while it remained stable in oxidative, hydrolytic neutral, thermal and photolytic conditions. The developed method was found specific as pure drug peak was separated from degradant. The resolution factor was found less than 2%**.** All degradant products were investigated by LC-MS (ESI).

Keywords: Opipramol, Force degradation, HPLC-MS, Method validation.

INTRODUCTION:

Opipramol 2-[4-(3-dibenzo[b,f]azepin-5-yl-propyl)-piperazin-1-yl]-ethanol Fig.1 is an associate tricyclic antidepressant and anxiolytic drug. It acts as a sigma receptor agonist having high affinity towards □1 receptor and occupancy of □1 receptor causes translocation of receptor to the peripheral areas of neuron, which further regulates neurotransmitter which causes release of the dopamine and serotonin. It is used in the treatment of general anxiety disorder, anxious-depressive state and somatoform disorders.[1]

Literature study shows High-Performance Liquid Chromatographic (HPLC) methods for determination of opipramol in human plasma,[2-3] Synthesis, characterization and pharmacological screening method of impurities,[4] Electroanalytical method for determination of opipramol in pharmaceutical preparations and biological fluids[5] and thin layer chromatographic method.[6] Furthermore, potential organic impurities in opipramol[7] were also determined. HPLC method for better sensitivity was reported [8] and also, most of the work have used liquid chromatography technique for better repeatability reliability, analysis time.[9] Existing literature indicates, there was no identification of degradant product by stress degradation study and development & validation of stability indicating HPLC-MS method. In this paper, the objectives of the study were, identification of degradant product produced by stress degradation and development & validation of stability indicating HPLC-MS method. So, we developed a rapid, accurate, reproducible and sensitive chromatographic stability indicating method of opipramol in bulk and pharmaceutical dosage form and identified its possible impurities by ESI-MS, which was validated in accordance with ICH guidelines.

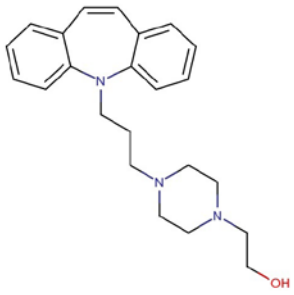

Fig. 1: Chemical structure of Opipramol

MATERIALS AND METHODS:

Drug and reagent:

Pure opipramol was obtained from Mylan laboratories, (Hyderabad, India). OPIPROL 50 marketed formulation obtained from Sun pharma laboratories ltd. (Assam, India). Analytical reagent grade sodium hydroxide, hydrochloric acid were purchased from thermosil fine chem industries (Mumbai, India). HPLC grade water, acetonitrile and triethylamine for chromatography were purchased from Merck Specialities Pvt. Ltd. (Mumbai, India). 3% hydrogen peroxide and ortho-phosphoric acid were purchased from Research-Lab Fine Chem Industries (Mumbai, India).

Instrumentation:

A quantitative HPLC system (Shimadzu Corporation, SPD-M20, Japan) equipped with LC solution software was used for LC studies, the detector was photodiode array detector having light source of deuterium (D2) and

tungsten (W) lamp with a wavelength range of 190-800nm. It had an on-line degasser containing binary pump and sample injector with 20 µL loop. Separation studies were carried out using C18 column.

Qualisil 5 BDS-C18 (250mm × 4mm i.d., 5µm particle size) (Netherlands). The stress degradation studies were carried out with the help of precision water bath (Lab hosp instrument and equipment, east Mumbai, India) which was assigned with thermostat for temperature control. Photo degradation studies were carried out in presence of photo-stability chamber (Thermostat scientific equipment Pvt. Ltd., Mumbai, India). Hot air oven (Bio technics India, Mumbai, India) was used for thermal study. The LC-MS system was controlled by HighStar 3.2 software and Bruker IMPACT-HD model was used. ESI was used as ionization source. The model had the mass resolution up to 50,000 FSR and mass detectable range from 100 to 3500 m/z. pH meter (Labman scientific instrument Pvt. Ltd., Chennai, India) was used to adjust the pH of the mobile phase and other solutions used during the study. Other instruments also used during the study were, Analytical balance (Contech instrument Ltd., Pune, India), Sonicator (Citizen digital ultra sonicator) and suction pump (rocker 300A vacuum filtration system).

Preparation of standard solution:

The standard solution of opipramol was prepared by dissolving 50 mg of opipramol in 100 ml of volumetric flask containing 40 ml of water. Further, it was sonicated for 10 min and then volume was made up to 100 ml using water to get approximately 500 µg/ml. 6 ml of standard stock solution was taken in 100 ml volumetric flask and made up to 100 ml with diluent (Acetonitrile: water,40:60) to get concentration of 30 µg/ml.

Preparation of sample solution:

20 film coated tablets of Opipramol were taken and powdered. Sample of powdered tablets equivalent to 30 mg of the active ingredient was weighed and transferred to 50 ml volumetric flask and mixed with 30 ml of diluent, was allowed to stand for 45 min with recurrent sonication for solubility. Then volume was made up to 50 ml to get concentration of 1000 µg/ml and then filtered through 0.45 µm membrane filter. Further, from stock solution, 1 ml solution was pipette out in 10 ml volumetric flask and diluted with diluents to get concentration of 100 µg/ml. Then the resultant solution was further diluted by taking 3 ml of solution from 100 µg/ml solution in 10 ml volumetric flask which was diluted with diluent to get concentration of 30 µg/ml.

Force degradation studies:

The drug was subjected to hydrolytic, thermal, oxidation and photolytic stress conditions. The force degradation studies were carried out on marketed drug substance to attain 5%-15% of degradation. For stress degradation extreme conditions i.e. 80°C and shorter time interval were set, so that the rate of degradation can be evaluated,[10] Stress degradation studies were carried out on opipramol dosage form as per ICH Q1A (R2).[11]

Hydrolytic degradation:

Hydrolytic degradation was performed in alkaline, acidic and neutral conditions. After required exposure all samples of stress conditions were diluted in presence of diluent to get final concentration of 30 µg/ml, before the injection. Samples were prepared by taking 3 ml of stock solution of drug substance (100 µg/ml) in 10 ml volumetric flask. In which 2 ml of hydrolytic agents i.e. 0.1 N HCL, 1 N NaOH and water were added. Further samples were kept at constant temperature of 80°C on water bath for 1 hour. After specified time interval samples were cooled by keeping at room temperature and subsequently samples were neutralised by using the same strength of acid and alkali as injected earlier into HPLC system.

Thermal degradation:

In thermal degradation study samples were subjected to dry heat in hot air oven for specific time interval where temperature was kept constant. Two drug samples each of 25.68 mg were taken in two 10 ml volumetric flasks and covered. One sample was kept in hot air oven for specified time interval with constant temperature of 60 °C and other was kept as control. Further sample solution of 30 µg/ml concentration were prepared by appropriately weighing sample, which was exposed to thermal stress and control state condition. Then samples were made up to the desired concentration with the help of diluent before proceeding for the HPLC analysis.

Oxidative degradation:

Sample was prepared by taking 3 ml of stock solution of drug substance (100 µg/ml) in 10 ml volumetric flask and adding 2 ml of 3 % H_2O_2 to it. Furthermore, sample was heated on water bath at 80 °C for 1 hour. The oxidative stress sample was diluted with diluent and subjected to HPLC analysis.

Photolytic degradation:

Photolytic degradation was carried out as per the ICH guideline Q1B.[12] The drug sample was spread across the petri dish evenly to form uniform layer having 2 mm thickness. The sample was exposed to light with overall illumination not more than 1.2 million lux hours and integrated near ultraviolet energy not less than 200-watt h/m^2 and control sample was wrapped in aluminium foil.[13] After the specified time interval of exposure, the samples were diluted further to achieve the concentration of 30 µg/ml for both stress sample and control sample. Thereafter, the samples were subjected for HPLC analysis.

Optimised Chromatographic condition and HPLC method development:

HPLC study were carried out on stress sample solutions in presence of control sample solutions by injecting each sample to achieve optimum resolution peak in the specific run time. The mobile phase consists of Ortho-Phosphoric Acid in 100 ml of water whose pH was adjusted to 3.26 with triethylamine (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B). Low pressure gradient mode was used to determine the Opipramol. Further, the mobile phases were filtered through 0.45 µm membrane filter with the help of Suction pump. The flow rate was set to be 1.0ml/min for low pressure gradient mode and injection volume set to 10 µL. Column temperature was ambient and run time was set to 10 mins. The eluent was monitored at 254nm. Before injecting the

drug solution, the column was washed and equilibrated for 45 mins with the mobile phase flowing through the system.

Method validation:

HPLC method validation process were carried as per the ICH guideline Q2 R1.[14] Method validation parameters that are covered in this study includes accuracy, precision, specificity, linearity and selectivity.

Linearity and range:

Linearity of an analytical method was determined by its ability to indicate that test results are directly proportional to the concentration of an analyte within a given concentration range from 10-70 µg/ml. Linearity study was carried out using solution containing 10-70 µg/ml of the drug and each linearity samples were injected in triplicate into the HPLC system.

Precision:

Precision of the method was established by using drug sample solutions of three concentrations: 20, 30 and 40 µg/ml. They were injected in triplicate form on the same day and on the next day. The standard deviation and percent relative standard deviation were calculated for both inter and intra-day precision. The repeatability of the system was determined by assaying the sample solution six times and relative standard deviation was calculated.

Accuracy:

The accuracy of the method was investigated by conducting recovery study with the help of standard addition method. The solutions were prepared by spiking pure drug into the tablet powder. Three individually prepared replicates of 80, 100 and 120 µg/ml of concentrations were analysed. Accuracy was done by determining the assay of samples and calculated the peak area responses of different samples by recovery method.

Specificity and selectivity:

Specificity of an analytical method was determined by stating the ability to define specific analyte of interest without any interference. Specificity study was done by developing the peak resolution factor, to distinguish between the drug peak from the other peaks. The selectivity of the method was investigated by studying peak purity of drug's peak with the help of PDA detector.

Limit of quantitation and limit of detection:

Based on the standard deviation and the slope, the limit of quantitation (LOQ) and the limit of detection (LOD) were calculated.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Ruggedness and Robustness:

The ruggedness of the method was investigated by reproducibility of test results by analysing samples. This was done by changing three different analysts. The robustness of the method was established by studying capacity of method to remain unchanged by small deliberate variation such as

1- Change in flow rate 1.0 to 1.2 ml

2- Change in injection volume from 10 to 15µL

Mass spectrometry study:

The mass spectra were recorded on Bruker impact II HD mass spectrometer. The analysis of the stress samples was carried out in positive electrospray ionization (ESI) mode having mass range of 100-3500 m/z using previously developed LC gradient method. The drug concentration of 30 µg/ml in diluent (water: acetonitrile, 60:40 v/v) was injected using syringe into the mass spectrometer. Mass study of hydrolytic and oxidative stress samples was done.

RESULTS AND DISCUSSION:

Method development and validation:

The desired separation was shown by the drug and its degradation products, done by using mobile phase as given above in low pressure gradient mode. The parameters such as linearity, range, system suitability, precision, accuracy, specificity, selectivity, LOD and LOQ were tested for validation of the liquid chromatography method. An appropriate linear response was seen at the selected drug concentration in the linearity study. The plot of peak area of sample with respect to concentration was linear in the range of 10-70 µg/ml. From the linearity study the correlation coefficient(*r*) and the slope were found to be 0.999 and 81445, respectively. The results obtained from linearity study for the concentration range of 10-70 µg/ml are shown in Table 1. The linearity data was used to establish the sensitivity of the method. From the linearity data, the LOD was found to be 2.4 µg/ml and LOQ was found to be 7.4 µg/ml. System suitability tests were carried out to find out retention time, theoretical plates, tailing factor and HETP. The suitability of the system was evaluated by studying various parameters given in Table 2. for repeatability the mean % RSD was found to be 1.07%. The mean % RSD values for inter-day and intra-day precision study were 0.998% and 0.954% respectively. Accuracy study was analysed on the basis of assay of the analyte component. Accuracy data is shown in Table 3. Assay of tablet dosage form of drug was performed by injecting sample solution (30 µg/ml) in triplicate form and amount recovered was 29.84 µg/ml, % assay 99.41 with % RSD 0.052. No interference of mobile phase was seen in sample and standard chromatogram. No excipient peak interfered in the sample study. So, the developed LC method was found to be specific. Peak purity index 0.9816, single point threshold 1.00 and resolution of 1.71 in alkali and 1.91 in acid of degraded products revealed the selectivity of the method. Robustness study were carried out and %RSD at two different flow rates was found to be 0.37% for 1.0 ml/min and 0.76% for 1.2 ml/min. Ruggedness study was done in presence of three different analysts and %R.S.D. was found to be 0.40%.

Degradation behaviour study:

Degradant sample of tablet dosage form of opipramol was susceptible to hydrolytic condition and stable at thermal, photolytic, neutral and oxidative conditions. Separation studies were carried out on reaction solution individually using C_{18} column. Chromatogram of opipramol reveals that the four degradation products were generated. Degradation products in Fig. 2 were obtained from acidic and alkaline stress condition. The degradation conditions and amount of degradant obtained are mentioned in Table 4.

Table 1: linearity data for opipramol

Conc.(µg/ml)	injection 1	injection 2	injection 3	average area	slope	correlation coefficient
10	1109534	1113689	1104950	1109391		
20	1836309	1830128	1832379	1832939		
30	2680842	2681745	2677901	2650163		
40	3401562	3395261	3399867	3398897	81445	0.999
50	4364841	4364008	4357841	4362230		
60	5147487	5156659	5146891	5150346		
70	5912498	5908018	5900198	5906905		

Table 2: system suitability parameters

	Retention time t_R (min)	Theoretical plates	Tailing factor	HETP
Average (n = 5)	5.073	7222.6	1.71	21.49
% RSD	0.986	11.63	0.8633	0.3064

t_r = retention time, HETP = Height Equivalent to Theoretical Plates, %RSD = percent relative standard deviation.

Table 3: recovery study of the method

level	Amt of sample added (µg/ml)	Amt of standard added (µg/ml)	Total conc.	Mean amount recovered (n = 3)	Mean % recovery	SD	%RSD
80%	30	24	54	23.96	99.87	0.835	0.836
100%	30	30	60	29.96	99.89	0.581	0.581
120%	30	36	66	35.95	99.89	0.749	0.746

Table 4: percentage of degradation observed in each of the stress conditions

Degradation type	Degradation condition	% Degradation
Acid hydrolysis	0.1 N HCl, 80°C, 1 h	9.5
Base hydrolysis	1 N NaOH, 80°C, 1 h	5.24
Neutral hydrolysis	H_2O, 80°C, 1 h	0.45
Oxidation	3% H_2O_2, 80°C, 1 h	1.05
Photolysis	1.2 million Lux h	0.87
Thermal	60°C, 24 h	0.65

Table 5: LC-MS data of DP's with possible molecular formulae

Degradant product	Theoretical masses	Best possible Molecular formulae	Experimental masses
DP-I	192.08	$C_{14}H_{11}N^-$	193.08
DP-II	205.11	$C_9H_{19}N_2OCl^-$	206.09
DP-III	170.14	$C_9H_{20}N_2O$	171.14
DP-IV	339.23	$C_{21}H_{29}N_3O$	340.25

DP = degradation product

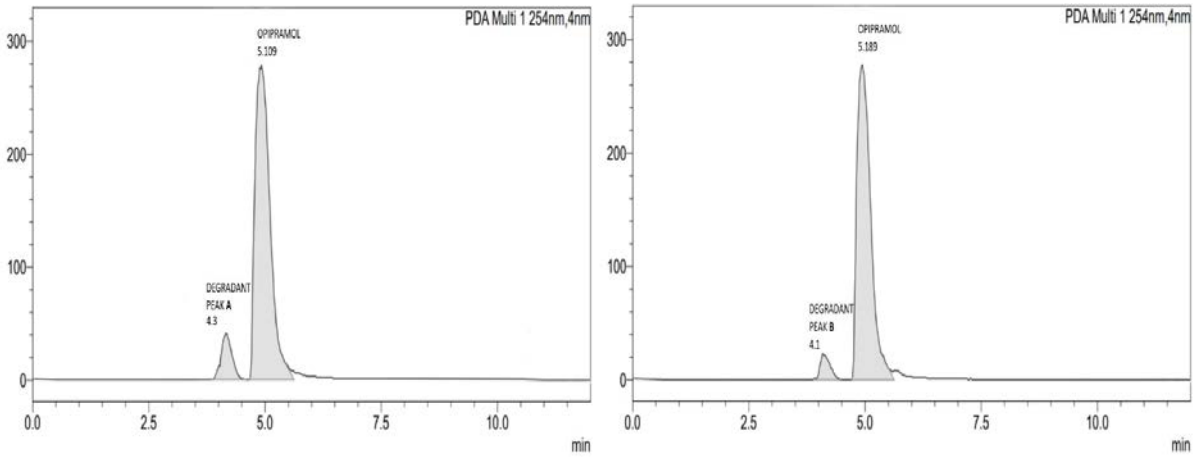

Fig. 2: HPLC-MS chromatogram of Opipramol degradant product in acidic(A) and alkaline(B) stress sample.

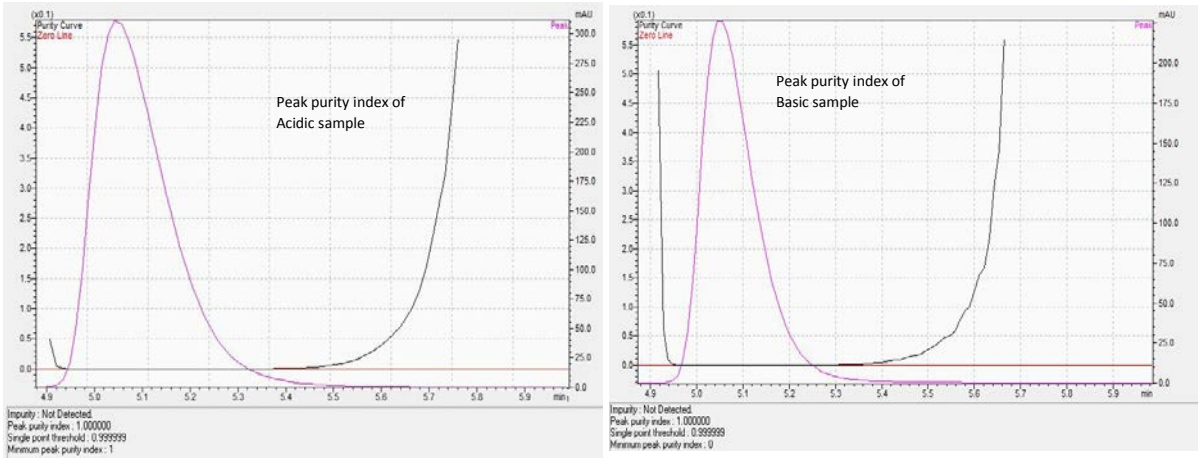

Fig. 3: peak purity index of Acidic and Basic stress sample

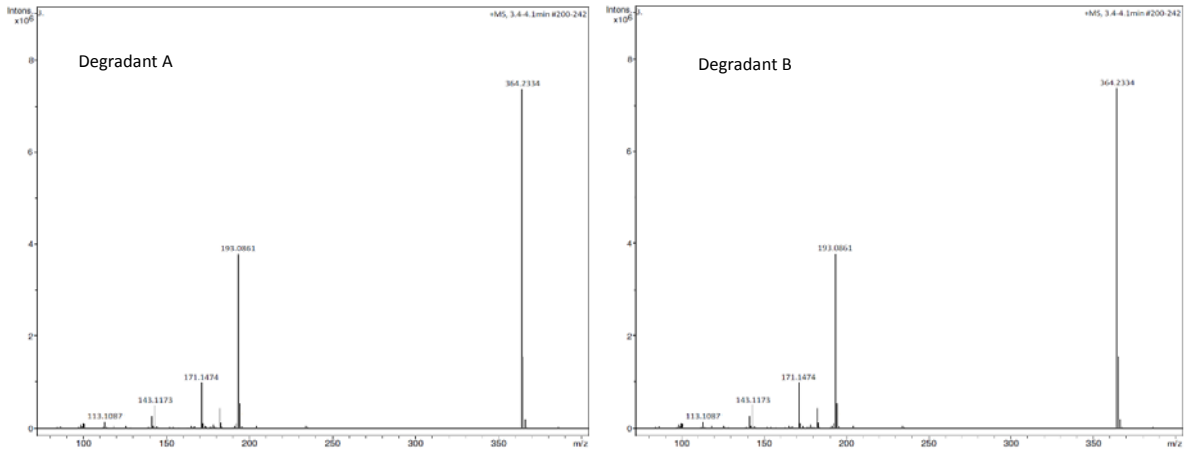

Fig. 4: Line spectra of degradation product.

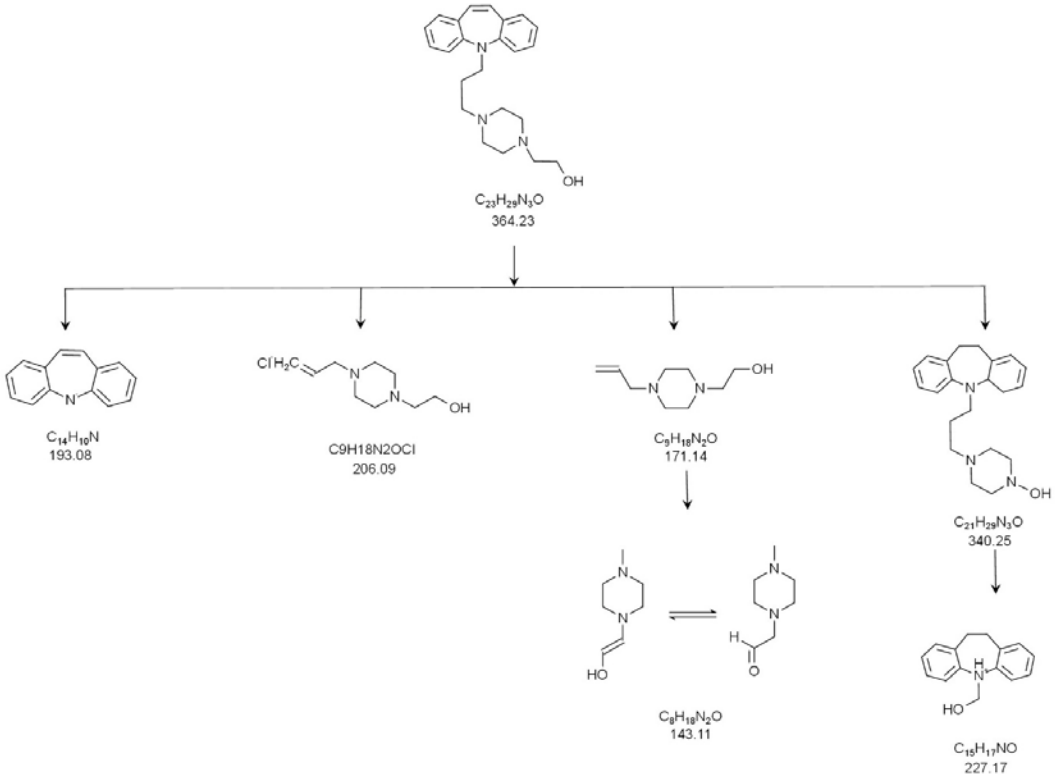

Fig. 5: Tentative degradation pathway of DPs under acidic and alkaline stress condition

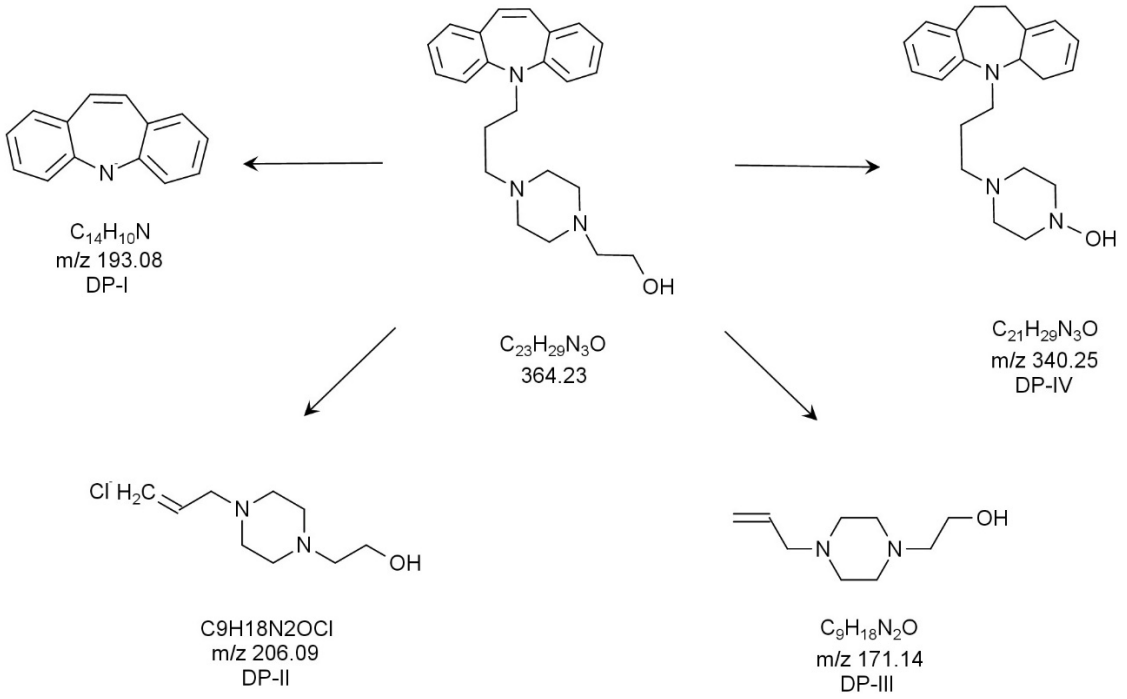

Fig. 6: Possible degradation product of opipramol

LC-MS study of stressed sample:

Mass spectra of all degradant products are shown in Fig. 4. The best possible molecular formulae, experimental masses and theoretical masses are mentioned in Table 5.

Identification of degradant products:

Further confirmation of the molecular structure was done with the help of mass spectra MS^+. The molecular ion peak $(M+H)^+$ and the base peak, of all compounds, were clearly obtained in mass spectral study. The molecular ion peaks were found to be in agreement with molecular weight of the respective compounds Fig. 5. The structural elucidation of obtained degradants was done on the basis of given LC-MS spectra.

DP-I (m/z 193.08):

The mass spectrum of degradant peak revealed that there was formation of major dibenzoazepine derivative compound in both acidic and alkaline condition. DP-I product's base peak with *m/z* 193.08 was formed as an 5H-Dibenzo[b,f]azepine after cleavage of aliphatic chain by hydrolysis. Whereas in alkaline condition the base peak with m/z 193.08 was formed after an attack of OH^- group on CH_2 aliphatic piperazine chain which gave 5H-Dibenzo[b,f]azepine moiety.

DP-II (m/z 206.09):

The mass spectrum of degradant peak shows that cleavage of C-N bond in acidic condition leads to formation of alkene side chain with *m/z* 206.09 and benzodiazepine due to protonation of nitrogen.

DP-III (m/z 171.14):

Based on the mass spectrum and the similarity of mass, hydrolysis of the C-N bond in presence of strong alkali leads to formation of DP-III with *m/z* 171.14 was found out. In the subsequent step, DP-III on cleavage of C-C bond gives fragment with *m/z* 143.11 which shows tautomeric structure.

DP-IV (m/z 340.25):

The mass spectrum of DP-IV revealed the formation base peak m*/z* 340.25 in both acidic and alkaline condition. Further ions with *m/z* 227.17 and *m/z* 210.14 were formed.

Degradation pathway of the drug:

Possible degradation pathways of the drug under various stress conditions is shown in fig. 6. DP-I was formed by cleavage of aliphatic piperazine chain by hydrolysis. DP-II was formed by hydrolysis of C-N bond. DP-III was obtained by cleavage of C-N bond in presence of acidic condition and DP-IV was formed by catalytic hydrogenation from the drug

CONCLUSION:

The developed HPLC-PDA detector method for the separation of opipramol and its degradation products was found specific, sensitive, selective, accurate, precise and robust. The developed method was validated as per ICH guideline. The % RSD was found less than 2%. The resolution factor was found less than 2% that indicated selectivity of the method. Stress degradation studies were carried out on opipramol in hydrolytic, oxidative, photolytic and thermal condition. The drug was labile in oxidative stress condition and stable in photolytic, oxidative and thermal conditions. The ESI LC-MS method was effectively developed to characterize degradants found in stress degradation studies. The study was found helpful in characterising four degradation products of opipramol based on LC-MS spectra. The experimental masses of DPs were found to be; DP1- 193.08, DP2-

206.09, DP1- 171.14 and DP1- 340.25. The tentative degradation pathway of opipramol was established by LC-MS study.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCE:

1. Mohapatra S., Rath N.M., Agrawal A., Verma J., Opipramol: a novel drug. Delhi Psychiatry J 2013; *16*:409-411.
2. Kees F., Bucher M., Mair G., Grobecker H., Determination of opipramol in human plasma by high-performance liquid chromatography with photometric detection using a cyanopropyl column. J. Chromatogr B 2001;*753(2):*337-42.
3. Lappenberg-Pelzer M., Tenczer J., Identification and determination of opipramol metabolites in plasma and urine. J. of Anal Toxicol 1998;*22(3):*215-9.
4. Mishra R., Siddiqui A.A., Rashid M., Ramesha A.R., Rohini R.M., Synthesis, characterization and pharmacological screening of various impurities present in opipramol, pargeverine and propiverine bulk drugs. Der Pharma Chemica 2010;*2*:185-94.
5. Turhan E., Uslu B., Electroanalytical determination of opipramol in pharmaceutical preparations and biological fluids. Anal Lett 2008;*41(11):*2013-32.
6. Butle S.R., Deshpande P.B., Development and validation of stability-indicating HPTLC method for determination of opipramol hydrochloride as bulk drug and in tablet dosage form. World J Pharm Res 2015;*4(09):*1952-1962
7. Luboch E., Wagner-Wysiecka E., Jamrȯgiewicz M., Szczygelska-Tao J., Magiełka S, Biernat J.F., Determination of the chemical structure of potential organic impurities occurring in the drug substance opipramol. Pharmazie 2010;*65(4)*:239-44.
8. Raju N.A., Ram M.J., Kumar V.K., Raju V.A., New high performance liquid chromatographic method for determination of opipramol in pharmaceutical dosage forms. Indo Am J Pharm Res 2016;*6(03)*:4587-4593.
9. Patel P.S., Maheshwari D.G., A review on chromatographic and spectrophotometric method for estimation of opipramol dihydrochloride in pharmaceutical dosage form. World J Pharm Res 2018;*7(5):*1522-1526.
10. Blessy M.R., Patel R.D., Prajapati P.N., Agrawal Y.K., Development of forced degradation and stability indicating studies of drugs-A review J Pharm Anal 2014 Jun 1;*4(3)*:159-65.
11. International Conference on Harmonization Steering Committee. ICH Harmonized Tripartite Guideline-Stability Testing of New Drug Substances and Products. ICH Q1A (R2). 2003 Feb 6.
12. International Conference on Harmonization Steering Committee. ICH Harmonized Tripartite Guideline-Photo stability testing of new drug substances and products. ICH Q1B. 1994 Nov 6.
13. Cione A.P., Tonhi E., Silva P., Stability indicating methods. Quality Control of Herbal Medicines and Related Areas Ed Yukihiro Shoyama 2011 Nov *4*:25-36.
14. ICH I. Q2 (R1): Validation of analytical procedures: text and methodology. International Conference on Harmonization, Geneva 2005