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SYNTHESIS, CHARACTERISATION OF IMPURITY PRESENT IN THE MANUFACTURE OF LOPRAZOLAM AND STUDY OF IMPURITY PROFILE BY HPLC

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ABSTRACT: The reaction of 5- (2- chlorophenyl)- 7- nitro- 3H- 1, 4- benzodiazepin-2-thione (I) with glycine (A) by means of Na_2CO_3 in refluxing ethanol in water gives 2-carboxymethylamino-7-nitro-5-(2-chlorophenyl)-3H-1, 4-benzodiazepine(II), which is cyclized by means of dicyclohexylcarbodiimide in methylene chloride to afford 8-nitro-(2-chlorophenyl)-1,2-dihydro-1H,4H-imidazo[1, 2-a] [1, 4]benzodiazepin-1-one (III). The reaction of (III) with dimethylformamide diethylacetal (B) by means of triethylamine in benzene yields 8-nitro-2-(dimethylaminomethylene)-6-(2-chlorophenyl)-1,2-dihydro-1H, 4H-imidazo[1,2a] [1,4]benzodiazepin-1-one(IV), which is treated with N-methylpiperazine (C) in refluxing toluene result 8-nitro-(2-chlorophenyl)-2-(N-methylpiperazin-1- ylmethylene)- 1, 2- dihydro- 1H, 4H- imidazo[1,2a] [1,4] benzodiazepin-1-one (V). This compound is finally treated with methanesulfonic acid. The condensation of 6-(2-chlorophenyl)-1,2-dihydro-8-nitro-1H, 4H-imidazo [1,2-a] [1,4] benzodiazepin-1one, with 1(dimethoxymethyl)-4-methylpiperazine (II) gives the free base of Loprazolam Mesylate (III), which is then treated with methanesulfonic acid. 5-aryl-1, 3-di-hydro-7-substitued-2*H*-1,4-benzodiazepine-2-thiones were condensed with glycine in aqueous ethanol to give the previously unreported amino acid. Dicyclohexylcarbodiimide in dry methylenechloride cyclized. These acids to the imidazolobenzodiazepines which were found to oil unstable to hydrolytic solvents.

INTRODUCTION: Impurity is defined as any substance, other than the substance of interest, coexisting such as starting material, intermediate or formed during the manufacture of the drug due to the side reactions [1]. Characterization and quantification of identified and unidentified impurities present in a drug substance is known as impurity profile. Impurities may arise in the final product by the following ways [2]:

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Impurities closely related to the product and coming from the chemical or biosynthetic route (during the formation of the bulk drug). Impurities formed due to spontaneous decomposition of the drug during the storage or on exposure to extreme conditions and the precursors may be present in the final product as impurities [3].

Acceptance Criteria of Impurities: Individual impurity not more than 0.1%, and total impurity not more than 1.0%. Impurities present in excess of 0.1% should be identified and quantified by sufficiently selective methods [4]. The suggested structures of the impurities can be synthesized and will provide final evidence for their structures previously determined by spectroscopic methods [5].

Therefore, it is essential to know the structure of these impurities in the bulk drug in order to alter the reaction condition and to reduce the quantity of impurity to an acceptable level [6]. Isolation, identification, and quantification of impurities help us in various ways, to obtain the pure substance with the least toxicity and contribute to the safety of drug therapy [7]. Quantitative determination of these impurities could be used as a method for quality control testing of every batch of the drug. Regulating authorities such as US FDA, CGMP, TGA, MCA insist on the impurity profiling of drugs. Hence studies are required to generate impurity profiles of drugs. Impurities may be classified into the following categories: Organic Impurities (Synthesis Related), inorganic Impurities and residual Solvents.

Characterization of Impurities: Once an impurity has been detected, it becomes necessary to estimate its content. If the estimations indicate that a given impurity content is greater than 0.1% then it must be characterized as per the FDA requirements. Hyphenated methods such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry [8] (LC-MS) or a number of other chromatographic-spectroscopic techniques are perfectly suitable for initial characterization of the impurities.

Analytical Methodologies:

Spectroscopic Methods	Separation Methods
Ultraviolet (UV)	Thin Layer Chromatography
Infrared (IR)	Gas Chromatography
Nuclear magnetic resonance (NMR)	High-Pressure Liquid Chromatography
Mass spectrometry (MS)	Supercritical Fluid Chromatography

Impurity Profile:

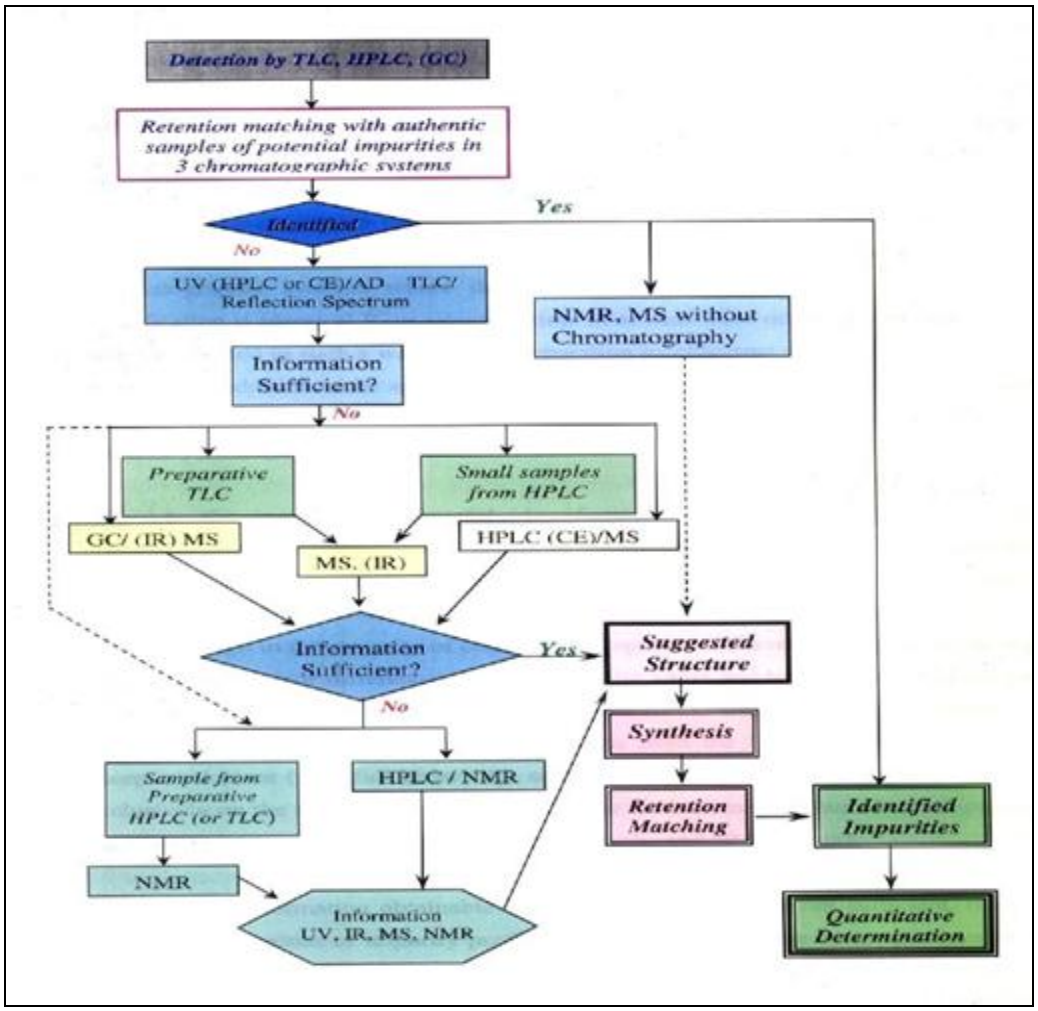


FIG. 1: A GENERAL SCHEME FOR DRUG IMPURITY PROFILING

As seen in the scheme, the procedure of impurity profiling begins with,

1. Detection of the impurities using the thin-layer chromatogram, high-performance liquid chromatogram or gas chromatogram.

2. Procurement of standard impurity samples from the synthetic organic chemists. These include the last intermediate of the synthesis products of predictable side reactions. degradation products if any.

3. These samples should undergo retention matching with the previously detected impurities in the chromatographic system where they were detected [9]. The criterion for positive identification is identical R_f or retention time in at least three different chromatographic systems.

4. In the case of unsuccessful identification with standard samples, the reasonable way to determine the structure of the impurity starts with the investigation [10] of the UV spectra, easily obtainable with the aid of the diode-array detector in the case of HPLC and quantification with the help of densitometer. If the information obtainable from the UV spectrum is not sufficient, the next step in the procedure of impurity profiling is usually to take the mass spectrum of the impurity [11].

   An advantage of the GC/MS method is that reliable molecular weight value is obtainable using chemical ionization and in addition, information of fragmentation, necessary for the solution of more complicated structure elucidation problems can also be obtained using the electron impact ionization technique.

   A disadvantage is that due to volatility and thermal stability problems, the Possibilities of this method are limited. An advantage of the HPLC/MS method is its general applicability. A disadvantage is, however, that the ionization techniques used in association with the generally used instruments (the older thermospray and the more up-to-date electrospray and atmospheric pressure chemical ionization [12] (APCI) techniques) usually give only molecular weight information.

5. The next step in impurity profiling is the synthesis of the material with the proposed structure. The retention and spectral matching of the synthesized material (impurity standard) with the impurity in question are carried out as outlined above.

   The possibilities of spectroscopic techniques in drug impurity profiling without chromatographic separation are also worth mentioning. Spectra obtained by using high-resolution, highly sensitive NMR spectrometers and mass spectrometers with electrospray/APCI facilities [13] are suitable to provide a fingerprint-like picture regarding the purity of the sample.

MATERIALS AND METHODS: 5-(O-chlorophenyl)-1, 3, dihydro-7-nitro-2H, 1, 4-benzo-diazepin-2-thione, Glycine, Sodium carbonate, Ethanol, N, N, Dicyclohexyl carbodiimide, Dimethyl ketal of N-formyl N-methyl piperazine. Triethylamine, Methane sulphonic acid, Chloroform, Ethanol, Acetone –L-R-Grade, Methanol-L-R-Grade, Hexane –L-R-Grade, Toluene-L-R; Grade, Ethyl acetate-l-R-grade, Isopropanol –L-R-Grade, Chloroform [14]. Ether, Aceto nitrile-L-R; Grade, Dichloromethane L-R-Grade, Isopropyl Ether.

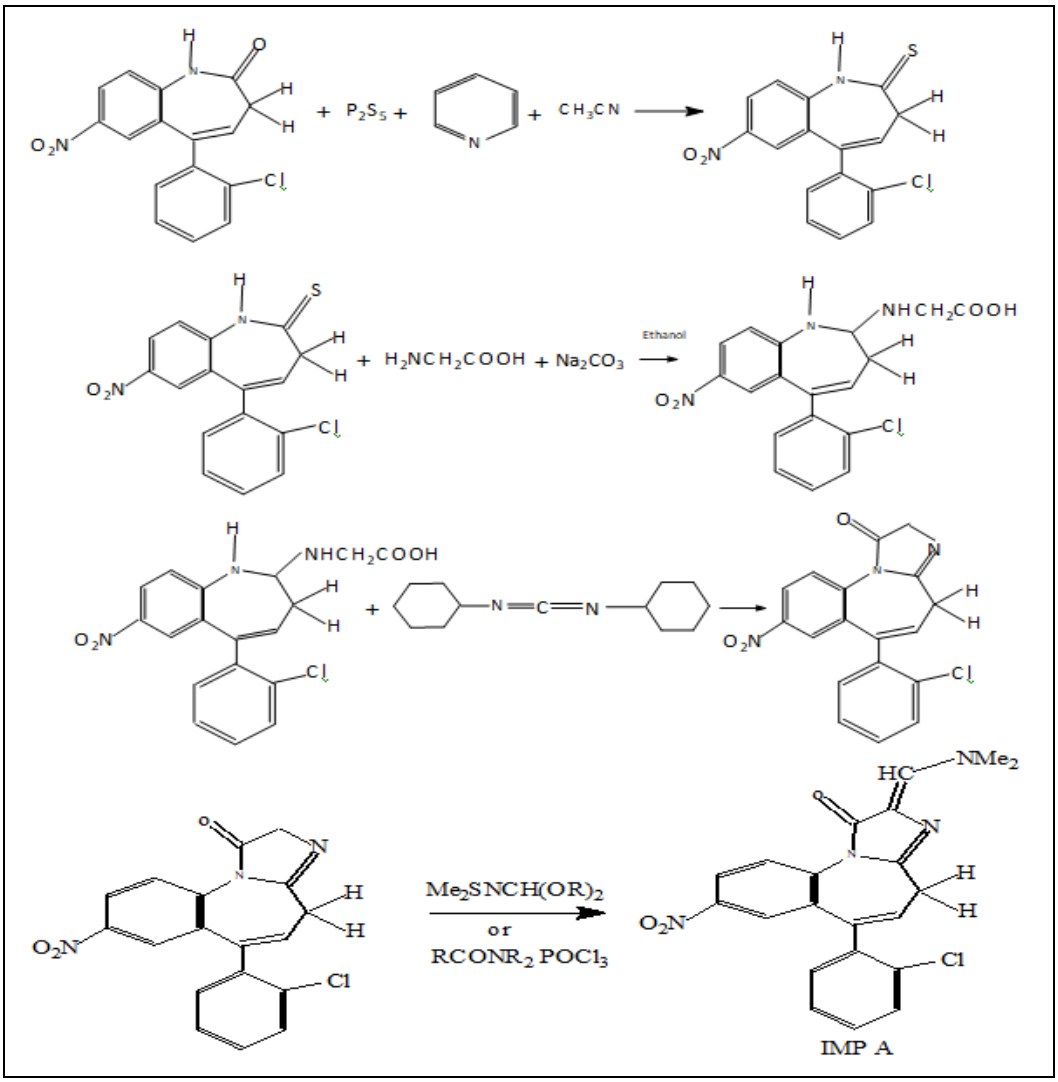
The synthetic work carried out in the R&D laboratory Nehru College of Pharmacy, Kerala, area.

IR, HPLC chromatograms are obtained from the QC department Lake Chemical and NMR spectra are obtained from the IISC Bangalore.

Instruments Used: Scientific Melting Point digital. Shimadzu FT IR8400S spectrometer. Cimarec Magnetic stirrer.

Parr pressure Hydrogenator. KEM UV chamber was used for the detection of spots during reaction monitoring. NMR spectra were recorded on a Bruker [15] spectrospin-400MHZ spectrometer using $CDCl_3$ as solvent and TMS as an internal standard.

Experimental Part:



Synthesis of 6-(2-chlorophenyl) 2, 4 dihydro [(dimethylamino) methylene] 8-nitro imidazo [1,2a] [1,4] benzodiazepin 1-one. (LPZM IMP A)

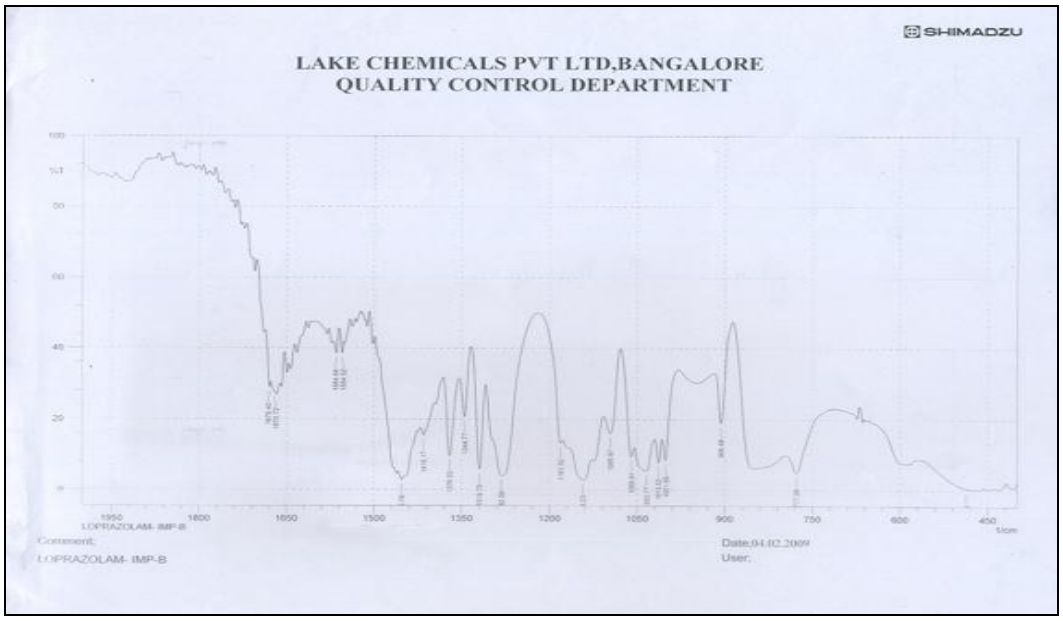


FIG. 2: IR SPECTRA OF IMPURITY-A

IR Spectrum: Functional Group, Wave Number (cm^{-1}): Aromatic c=c stretching. 1577-1620 cm^{-1}, Aromatic C-H bend, 775 cm^{-1}, Aliphatic C-H bend, 2937 cm^{-1}, Aliphatic C=C, 1680-1620 cm^{-1}, C-N stretching, 1350 cm^{-1} Aromatic C-NO_2, 1540-1340 cm^{-1}, C=O, 1600-1650 cm^{-1}, C-Cl, 748 cm^{-1}.

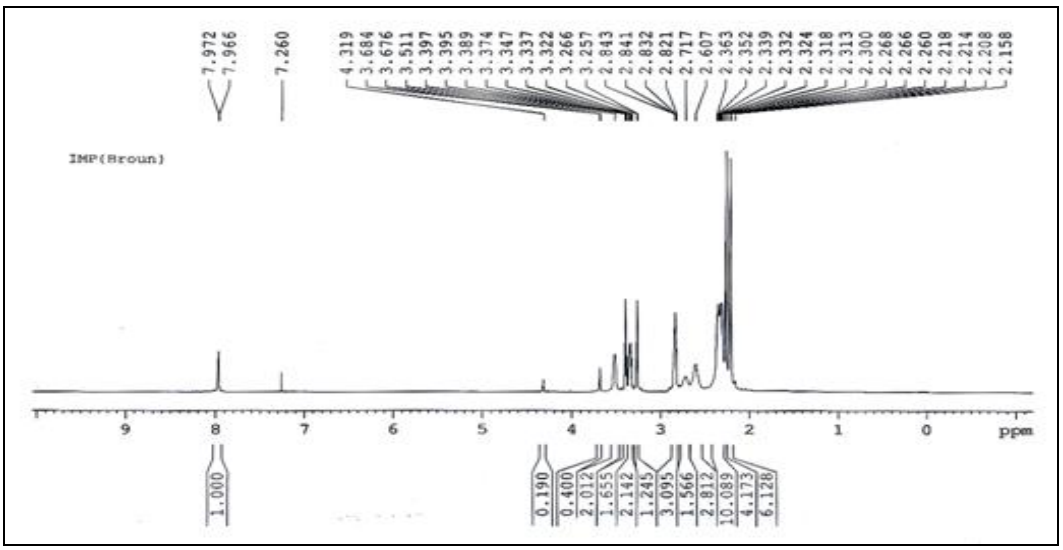
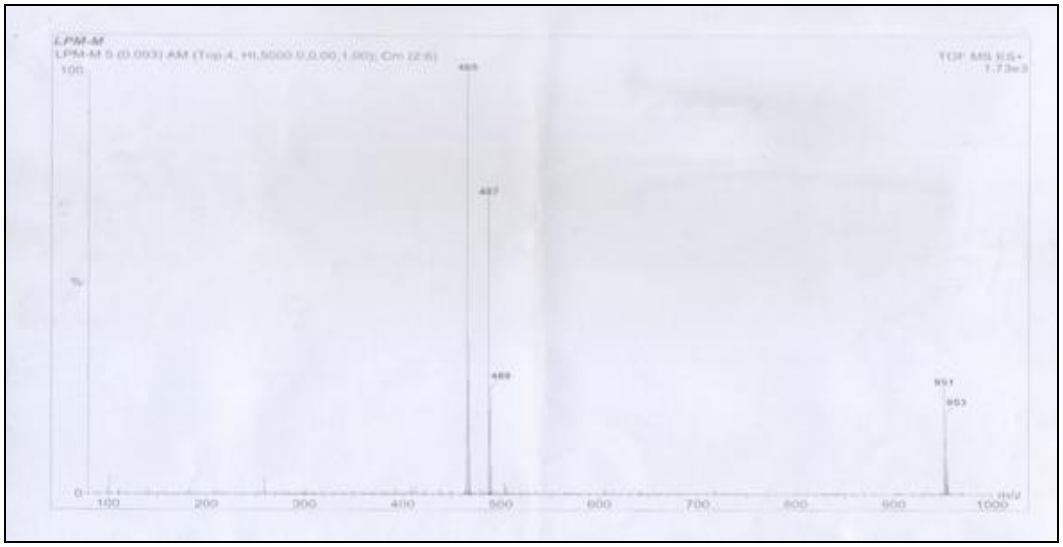


FIG. 3: NMR SPECTRUM DETAILS OF IMPURITY-A

3H, s, CH_3 -2.6, 8H, m, CH_2-2.30-2.36, 3H, s, OCH_3-2.86, - 3.5, 3H, S, OCH_3-3.26

Mass Spectra: According to IR, ^1H NMR spectral data obtained, the structure of the molecule can be written as.



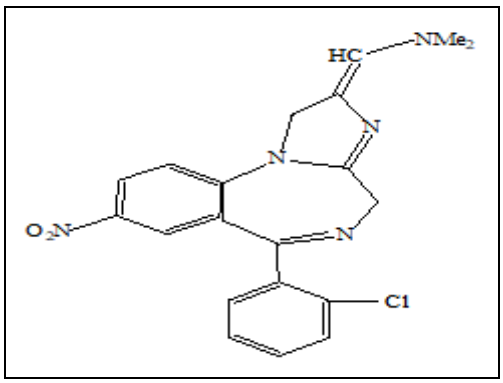
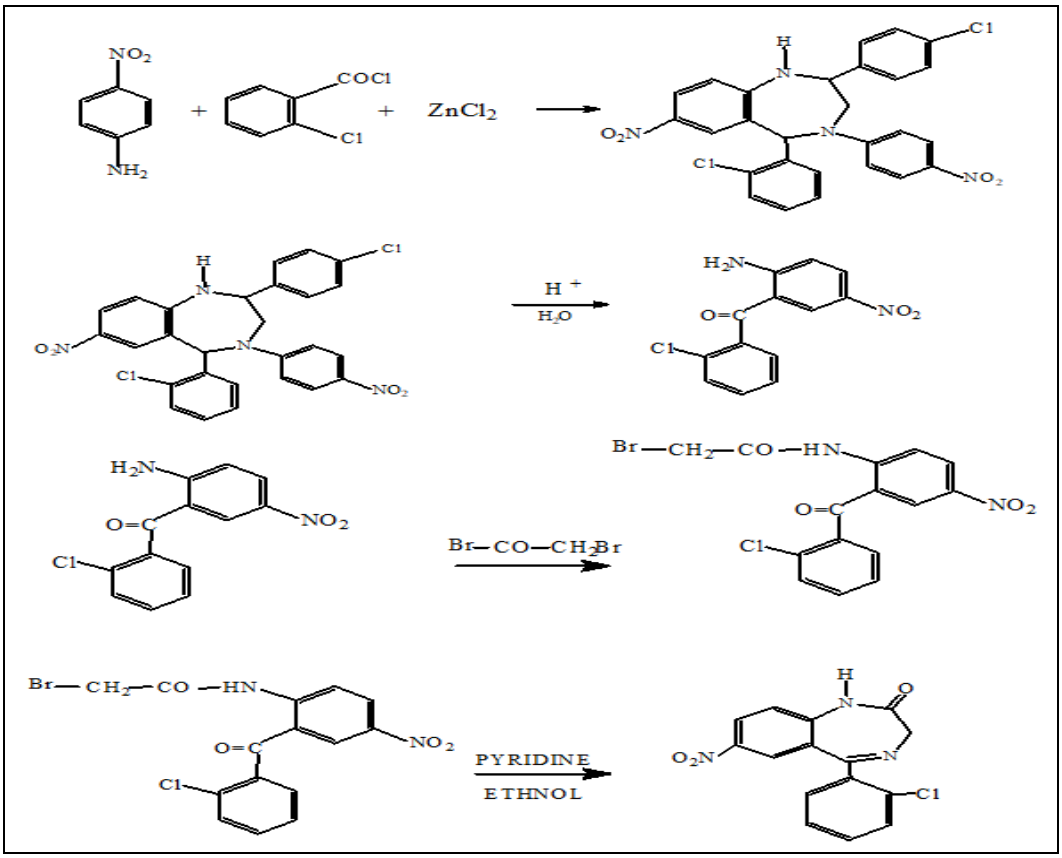
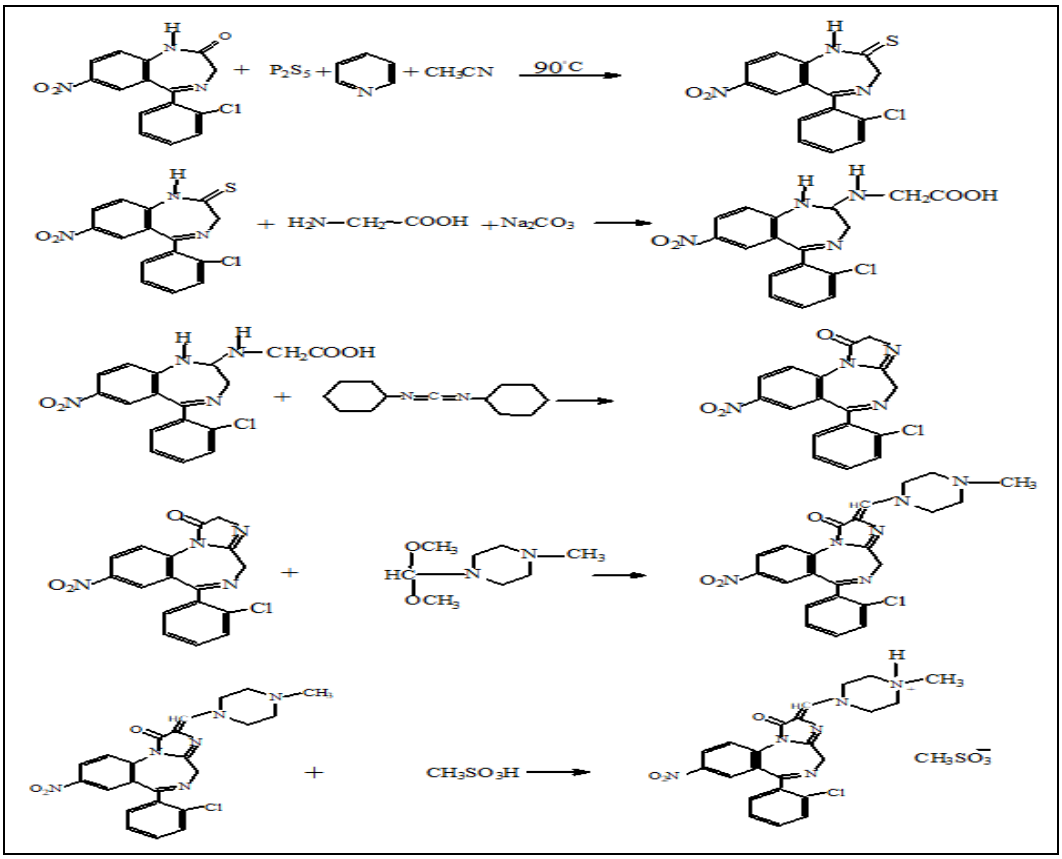


FIG. 4: MASS SPECTRA OF IMPURITY-A

Synthesis of 5(2-chloro phenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one:



Synthesis of Loprazolam Mesylate



Synthesis of 5-(2-chlorophenyl)-1,3-dihydro-7-nitro 2H-1,4 benzodiazepine-2-one

Ortho chlorobenzoyl chloride is reacted with para-nitro aniline in modified friedel craft reaction to yield 2-amino-5-nitro-2-chlorobenzophnone. The amino-ketone is then condensed with bromo-acetyl bromide to form 2-bromo acetamido-5-nitro-2-chloro benzophenone [16]. This compound is isolated and converted to the corresponding acetamido compound by reacting it in solution with ammonia. The ammonium bromide byproduct is separated and the solvent removed.

The residue was taken up in 5N anhydrous hydrogen chloride in methanol to form hydrochloride salt, which is then taken up in boiling ethanol. Pyridine [17] was added which catalyzed ring closure to get 5-(2-chloro phenyl)-1, 3- dihydro- 7- nitro 2H- 1, 4- benzodiazipin-2-one. The yield was 70%.

Synthesis of 2-carboxy-methyl amino-7-nitro-5 (o-chlorophenyl)3-H[1,4]benzodiazepine: Into 3 litres of de-mineralized water are introduced 1.36 kg of glycine followed, gradually, by 1.450 kg of sodium carbonate. The resultant mixture is agitated for 30 min at ambient temperature and the 15 liters of ethanol are introduced [18] therein followed, over about 5 min by 3 kg of 5-(o-chlorophenyl)-1,3 dihydro-7-nitro-2H,1,4 –benzodiazepin -2-thione. The mixture obtained is taken to reflux for 30 min and then distilled under reduced pressure. 15 liters of demineralized water are then added in two portions followed by 7.5 liters of DCM phases are subsequently separated.

The aqueous phase is re-extracted with 3 × 6 liters of DCM then the chloromethylinic [19] phases are washed with water and all the aqueous phases are combined. 18 liters of methylene chloride are added thereto followed at 0 to 5°, by 1.3 liters of 20 N HCl. Chloromethylenic solution of 2- carboxy methyl amino-7-nitro-5(o-chlorophenyl)3-H-[1,4] benzodiazepine is obtained and used directly in the following stage.

Synthesis of 8–nitro-1, 2-dihydro-6-(o-chlorophenyl)-1H, 4H-imidazole [1,2-a][1,4] benzodiazepine -1-one: To the chloromethylenic solution of 2-carboxy methylamino7-nitro5-(o-chlorophenyl)3H- [1, 4]- benzodiazepine obtained above is added, at 5° cover about 2 min, a solution of 1.865 kg of dicyclohexylcarbodiimide in 3 liters of methylene chloride. The mixture obtained is agitated for 40 min, left to stand for one night then separated [20]. The resultant product is washed twice with 3 liters of methylene chloride and the chloromethylenic solution of 8 –nitro-1,2-dihydro-6-(o-chlorophenyl)-1H, 4H-imidazole [1,2-a][1,4] benzodiazepine -1-one. TLC was checked by reaction mixture in DCM and mobile phases were Methanol and DCM.

Synthesis of 8-nitro 1,2-dihydro 2-(N-methyl piperazine-1-yl)-methylene-6-(o-chlorophenyl)-1H,4H-imidazole [1, 2 a] [1, 4] benzodiazepine-1-one: To the methylenic solution of 8 -nitro-1,2-dihydro-6-(o-chlorophenyl)-1H,4H-imidazole [1,2-a][1,4] benzodiazepine -1-one obtained above are added, over about 5 min and at ambient temperature, 650 gm of triethylamine [21] followed by 2.4 kg of a solution of dimethyl ketal [22] of N-formyl –N-methyl piperazine [23] [prepared by addition of 3.3 kg of N-methyl piperazine in to 2 kg of dimethyl ketal of dimethylformamide (DMF-DMA).

The mixture obtained is taken to reflux for 15 h. The uncombined N-methyl piperazine is subsequently distilled off under reduced pressure and the remainder is agitated for 1 h under reduced pressure at 115-120 °C then cooled to 20 °C. 3.920 kg of the brown solution of dimethyl ketal of N-formyl N-methyl piperazine is obtained.

The resultant mixture is agitated for 1 h 30 min and then concentrated to dryness under reduced pressure. 6 liters of ethanol are added to the residue and the mixture obtained is distilled, the volume being kept constant by the addition of ethanol until vapors are obtained at 78 °C.

The mixture cooled at 0° to 2°C agitated for 2 h [24] and separated. The recovered product is washed with ethanol. 4.260 kg of crude products is collected and purified by treating with active charcoal than with ethanol.

Finally, 3.362 kg of 8-nitro 1,2-dihydro 2-(N-methyl piperazine- 1- yl)- methylene- 6- (o-chlorophenyl)-1H, 4H-imidazole [1, 2-a] [1, 4] benzodiazepine-1-one. Which treated with 88 gm methanesulfonic acid to got 4.80-gram Loprazolam Mesylate [25] mesylate. The yield was 75%.

IR Spectral Data of Loprazolam Mesylate:

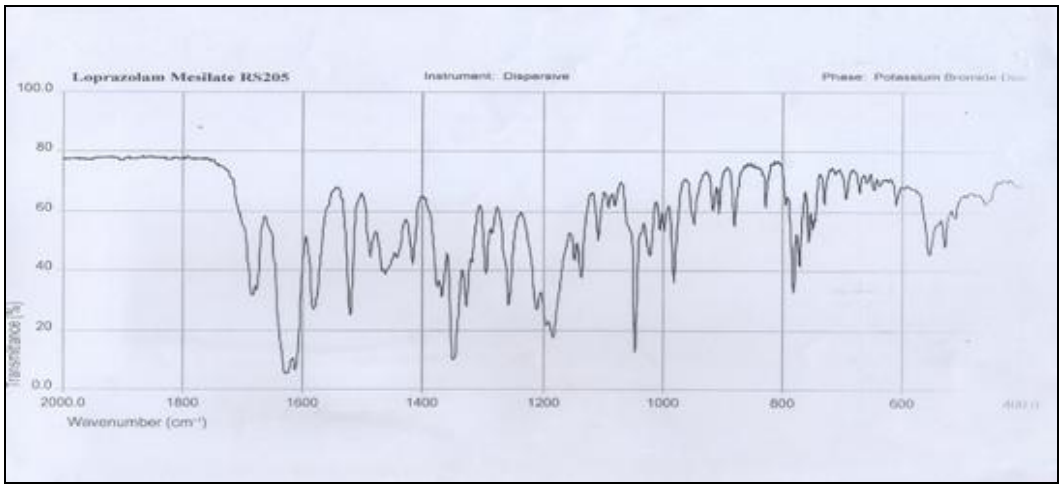


FIG. 5: IR SPECTRAL DATA OF LOPRAZOLAM MESYLATE

C=C stretching- 1650-1577 cm^{-1}, C-Cl -748 cm^{-1}, C-C=C- 1500-1450 cm^{-1}, C=O-1650 cm^{-1}, C-H aliphatic-2937 cm^{-1}, C=CH- 800-900 cm^{-1}, C=N stretching- 1600-1700 cm^{-1}

Protone NMR:

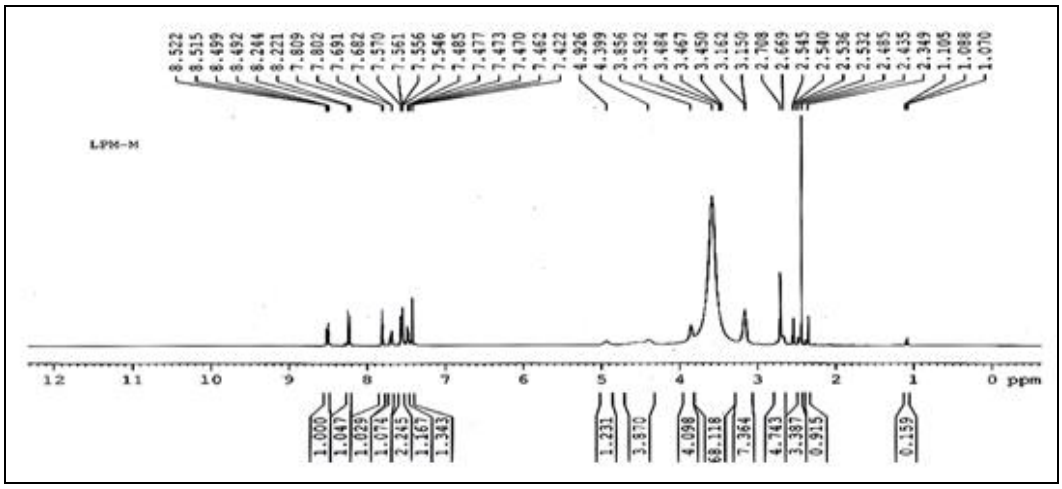


FIG. 6: PROTONE NMR

2H, s, CH_2- 4.9 ppm, 5H, m, (aromatic H)- 7.4-7.6 ppm 1H, s, (Aromatic H)- 7.8 ppm 1H, s, CH- 3.8 ppm, 8H, m, CH-2.5-2.7 ppm

Mass Spectra:

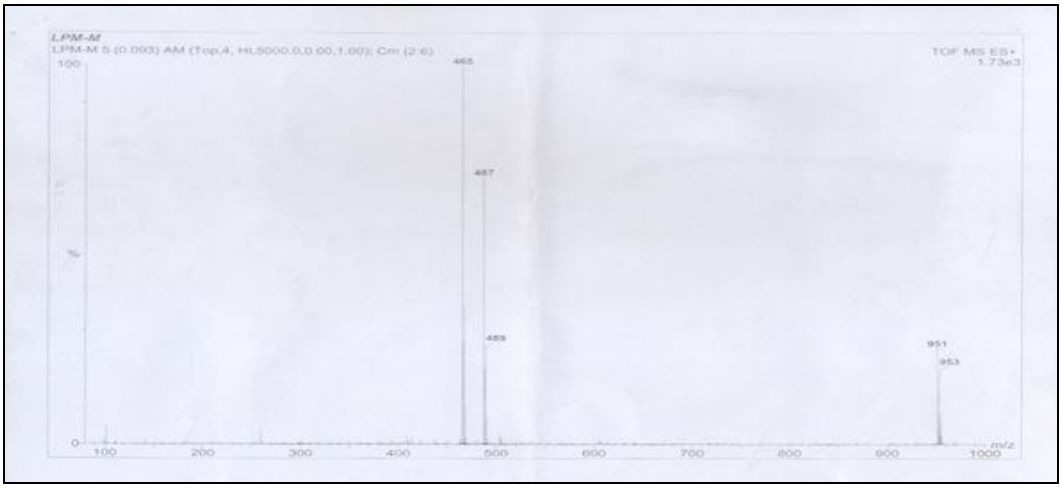
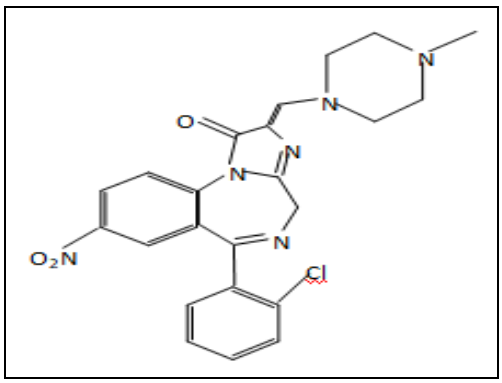


FIG. 7: MASS SPECTRA

According to IR, ^{1}H NMR, IR, and MASS spectral data obtained, the structure of the molecule can be written as



Analytical Data: Development of the HPLC [17] method for the identification and quantification of synthesized impurities of Loprazolam Mesylate.

Selection of Column: C8 size; 1=0.25m, Ø=4.0mm.

Selection of Mobile Phase: The selection of mobile phase was chosen taking [26] dipotassium hydrogen phosphate buffer pH adjusted to 3.8 by using dilute ortho-phosphoric acid and acetonitrile and methanol (71.45:20.7:7.85).

λ_{max} Determination: λ_{max} was found to be 200.05

Flow Rate: Flow rate was set to 1 ml per minute throughout the process.

Materials: Loprazolam Mesylate, Impurities (synthesized and characterized in lab) Di-potassium hydrogen phosphate (IR grade) Orthophosphoric acid (IR grade), Acetonitrile (HPLC grade), Methanol (HPLC grade), Distilled water (HPLC grade).

Instrument: The L.C. consists of Shimadzu 10 ATVP pump, Rhedyn injector fitted with 20 µl loop and a 10 AVD UV-Visible detector. The output was monitored and integrated using CLASS-VP software.

Mobile Phase: Di-Potassium hydrogen phosphate, Buffer; Acetonitrile.

Mix 71.45 volumes of a solution [27] containing 4.34 g/ml Dipotassium Hydrogen Phosphate buffer pH adjusted to 3.29 using Orthophosphoric acid, which 20.7 volumes of Acetonitrile and 7.85 volumes of methanol .the mobile phase was filtered through a 0.45-micrometer Nylon membrane filter and degassed by sonicating for about 15 min prior to use.

Dipotassium Hydrogen Phosphate Buffer: Dissolve 2.17 gm of Dipotassium hydrogen phosphate in 450 ml distilled water, pH adjusted to 3.29 with dilute orthophosphoric acid, and the volume up to 500 with distilled water.

Chromatographic Conditions:

Column: C 8 Size; 1=0.25m, Ø=4.0mm

Detector: U-V-200.5(Abs)

Retention time: 13.387

Procedure:

Preparation of Standard Solution of Loprazolam Mesylate: 12.5 mg slandered Loprazolam Mesylate was dissolved in little quantity of methanol. Then the solution was diluted up to 15 ml methanol [28].

Individual injection of different impurity A, and Loprazolam Mesylate.

Preparation of Stock Solution of Individual Impurity [29] Solution: 5.0 mg of each impurity of A, and Loprazolam Mesylate were exactly weighed and dissolved in about 5 ml of methanol in 10 ml volumetric flask. The volumetric flax was sonicated for 5 min and volume was made 10 ml with methanol. The resulting solution was about 0.5 mg per ml. The individual sample was injected separately (20 µl), and retention [30] time was recorded for each sample, and the stranded reference solution [31] was injected and retention time was a check.

Separation of Mixture of Impurities: From the above stock solution 1 ml of each impurity was pipetted out into a 25 ml volumetric flask and volume was made up to 25 ml with the standard reference solution. The mixture was sonicated for five mints, and 20 µl of the solution was injected and retention time was recorded [32].

The solution of standard Loprazolam Mesylate and its impurities were injected and chromatograms were recorded for three consecutive injections.

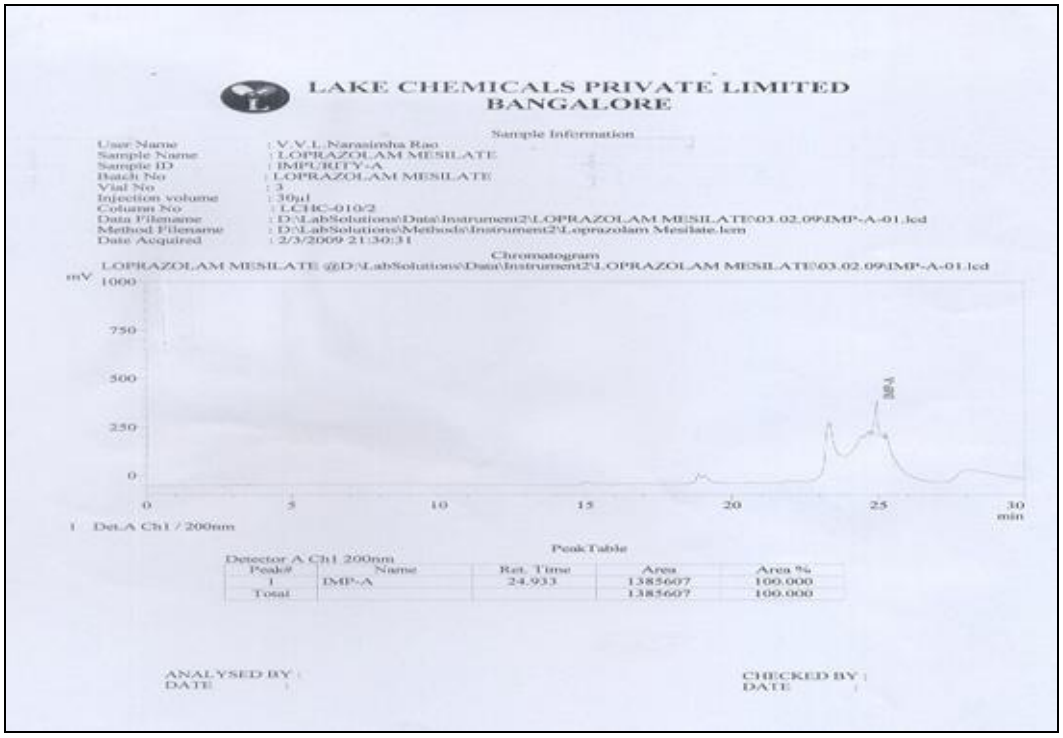


FIG. 8: HPLC OF IMP A

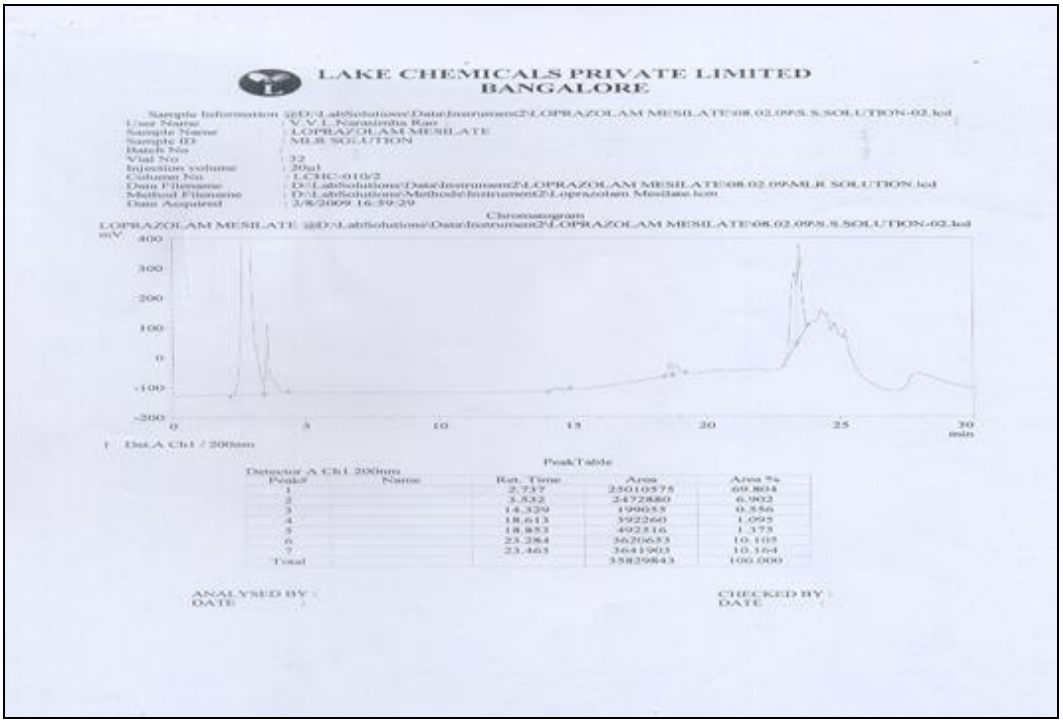


FIG. 9: HPLC OF LOPRAZOLAM MESYLATE MESYLATE

Identification and Characterization: On synthesizing a new compound its is to be identified by means of physical or chemical parameters like melting point, boiling point, solubility [33], chemical tests elemental analysis, *etc*. Other analytical methods that were also applied are TLC, UV, IR, NMR and Mass spectroscopy, *etc*. a brief outline of which are given below.

Melting Point: The melting points of synthesized pure compounds were carried out using Theil's tube methods [34].

Thin Layer Chromatography: The technique is widely used for the identification of organic compounds with characteristic R_f values. After the development of chromatogram on prepared silica

gel plates were used appropriate mobile phase, the spots were detected by placing the plate in the UV chamber.

Infrared Spectroscopy: Infrared spectroscopy is one of the most important tools for determining the various functional groups and possible chemical structures. This technique is based upon the molecular vibration of the compound such that each and every bond will vibrate at different frequencies and this vibration frequency corresponds to the IR frequency.

Nuclear Magnetic Resonance Spectroscopy: The interaction between matter and electromagnetic forces can be observed by subjecting a substance simultaneously to two magnetic forces, one stationary and other varying at some radio-frequency. At a particular combination of fields, energy is absorbed by the sample, and absorption can be observed as a change in signal developed by a radiofrequency detector and amplifier.

Mass Spectroscopy: This technique is useful in providing information regarding atomic and molecular weights, structure, mechanism, the kinetics of the reaction and mixture analysis. This technique involves bombardment of electrons and converted to highly energetic positively charged ions, which can break up into smaller ions and sorting them in the gas phase, into a spectrum according to into their mass/charge ratio.

CONCLUSION: The main objective of this study is to synthesize and characterize the impurities, which are formed during the manufacture of Loprazolam Mesylate and to develop an HPLC method for the identification of these impurities present in this bulk drug. IMP-A: 6-(2 Chlorophenyl)-2,4 diehydro-2-[(dimethylamino) methylene] 8- nitroimidazo [1,2 a] [1,4] benzo diazephine 1-one. HPLC method has been developed for the separation of the related impurities of Loprazolam Mesylate. The developed method gave a good separation with a good resolution of more than 1.15 and with good peak symmetry. Only MLR impurity can't be separated by this HPLC method.

The various synthesized impurities were identified and characterized by TLC- By using Hexane: Ethyl acetate = 3; 2.

I.R-SHIMADZU FTIR 8400S Spectrometer using KBR pellet method, Nuclear Magnetic Resonance Spectroscopy: The NMR spectral analysis of compounds was carried in a Bruker spectrospin-200 NMR spectrophotometer at IISc, Bangalore. The solvent used was $CDCl_3$ and DMSO. Mass spectra-SHIMADZU GC/MS SPECTROMETER 210.

ACKNOWLEDGEMENT: The authors are thankful to the Chemistry Department Nehru College of Pharmacy Kerala.

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES:

1. Svenson S and Tomalia DA: Dendrimers in biomedical application-reflections on the field. Adv Drug Deliv Rev 2012; 64: 102-15.
2. Kobayashi H and Brechbiel MW: Dendrimer-based macromolecular MRI contrast agents: characteristics and application. Molecular Imaging 2003; 2(1): 15353500200 303100
3. Hummelen JC, Van Dongen JL and Meijer EW: Electrospray mass spectrometry of poly (propylene imine) dendrimers-the issue of dendritic purity or polydispersity. Chem-Eur J 1997; 3(9): 1489-93.
4. Tam JP: Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proc Natl Acad Sci 1988; 85(15): 5409-413.
5. Ota S, Ono T, Morita A, Uenaka A, Harada M and Nakayama E: Cellular processing of a multi-branched lysine core with tumor antigen peptides and presentation of peptide epitopes recognized by cytotoxic T lymphocytes on antigen-presenting cells. Can res 2002; 62(5): 1471-76.
6. Shinde A: Solubilization of poorly water-soluble drugs. Pharm info. Net 2007; 5(6): 44-52.
7. Ringel I and Horwitz SB: Studies with RP 56976 (taxotere): A semisynthetic analog of Taxol. J Natl Cancer Inst 1991; 83(4): 288-91.
8. Marshall CR, A Noor, Vincent JB, AC Lionel, Feuk L, Skaug J and Thiruvahindrapduram B: Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 2008; 82(2): 477-88.
9. Sharma P, Denny WA and Garg S: Effect of wet milling process on the solid-state of Indomethacin and Simvastatin. Int J Pharm 2009; 380(1-2): 40-48.
10. Mannens G, Meuldermans W, Snoeck E and Heykants J: Plasma protein binding of risperidone and its distribution in blood. Psychopharmacology 1994; 114(4): 566-72.
11. Bakhbakhi Y, Charpentier PA and Rohani S: Experimental study of the GAS process for producing micro particles of Beclomethasone-17, 21-dipropionate suitable for pulmonary delivery. Int J Pharm 2006; 309(1-2): 71-80.
12. Saari SM, Vidgren MT, Koskinen MO, Turjanmaa VM, Waldrep JC and Nieminen MM: Regional lung deposition and clearance of 99m Tc-labeled Beclomethasone-DLPC liposomes in mild and severe asthma. Chest 1998; 113(6): 1573-79.

13. Darwis Y and Kellaway IW: Nebulisation of rehydrated freeze-dried beclomethasone dipropionate liposomes. Int J Pharm 2001; 215(1-2): 113-21.
14. Gautam SP and Verma A: PAMAM dendrimers: Novel polymeric nanoarchitectures for solubility enhancement of candesartan Cilexetil. Pharm Sci 2012; 1: 1-4.
15. Fujii M, Hori N, Shiozawa K, Wakabayashi E, Kawahara E and Matsumoto M: Effect of fatty acid esters on permeation of Ketoprofen through hairless rat skin. Int J Pharm 2000; 205(1-2): 117-25.
16. Gallicano K and Peloquin C: Comparative pharmacokinetics and pharmacodynamics of the Rifamycin antibacterials. Clinpharmacokinet 2001; 40(5): 327-41.
17. Singh I, Kaur KJ, Bhade S, Kaul CL and Panchagnula R: Bioequivalence trials of Rifampicin containing formulations: extrinsic and intrinsic factors in the absorption of rifampicin. Pharmacol Res 2004; 50(3): 317-27.
18. Perrin MA and Leveiller F: Docetaxel: solid-state characterization by X-ray powder diffraction and thermal gravimetery. J PHYS IV 2001; 11(PR10): Pr10-221
19. Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill 1996.
20. Charpentier PA and Rohani S: Experimental study of the GAS process for producing micro particles of Beclomethasone-17, 21-dipropionate suitable for pulmonary delivery. Int J Pharm 2006; 309(1-2): 71-80.
21. Vidgren MT, Koskinen MO, Turjanmaa VM and Nieminen MM: Pulmonary distribution and clearance of two Beclomethasone liposome formulations in healthy volunteers. Int J Pharm 1999; 181(1): 1-9.
22. Najlahc M, D' Emanuele A and Elhissib A: PAMAM dendrimers as aerosol drug nanocarriers for pulmonary delivery via nebulization. Int J Pharm 2014; 461: 242-50.
23. Cyclooxygenase-2 inhibitors in gynecologic practice. Clin Med Res 2003; 1(2): 105-10.
24. Vijayarajkumar P: PEGylated nanoarchitecture mediated solubility enhancement of tyrosinekinase inhibitor. Ach Sci Res 2015; 3: 119-22.
25. Drugs used in the chemotherapy of tuberculosis, Mycobacterium avium complex disease, and leprosy. The pharmacological basis of therapeutics, 10th Ed. McGrawHill, New York, NY 2001; 1273-94.
26. AP Guimarães, MA Pacheco, Dias DM, Furtado VR, de Alencastro RB and Horta BA: Association of the anti-tuberculosis drug Rifampicin with a PAMAM dendrimer. J Mol Graph Mode 2015; 60: 34-42.
27. Patel R, Patel H and Patel PM: Triazine based dendrimer as solubility enhancers of ketoprofen: effect of concentration, pH and Generation. Int J Pharm Pharm Sci 2014; 6: 357-61.
28. Saper J, Silberstein S and Sheftell F: Efficacy and safety of acetaminophen, aspirin and caffeine in alleviating migraine headache pain: three double-blind, randomized, placebo-controlled trials. Arch Neur 1998; 55(2): 210-17.
29. Driessche IV, Hoste S, De Smedt S, Demeester J and Remon JP: An oral controlled release matrix pellet formulation containing nano crystalline Ketoprofen. Int J Pharm 2001; 219(1-2): 81-87.
30. Chermann JC, Colliec-Jouault S, Sinquin C, Simon G, Cerantola S, Riadi H and Bourgougnon N: Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (Rhodophytha, gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, Ceramiales). Marine Drugs 2011; 9: 1187- 09.
31. Pabst MJ and Jakoby WB: Glutathione-Stransferase the first step in mercapturic acid formation. Journal of Biological Chemistry 1974; 249: 7130-39.
32. Kim YH, Park WS, Ahn WG, Park OK, Kwon SH, Morita K, Shim I and Her S: Novel antidepressant-like activity of propolis extract mediated by enhanced glucocorticoid receptor function in the hippocampus. Evidence-Based Complementary and Alternative Medicine 2013; 1-10.
33. Sharida F, Raudzah AR, Shamima AR and Apryani E: Antidepressant like effect of mitragynine isolated from *M. speciosa* Korth in mice model of depression. Phytomedicine 2011; 18: 402-7.
34. Zhang XL, Dong J, Yang J, Zhang YL, Ning QF, Shan XW and Li Y: Venlafaxine ameliorates the depression like behaviors and hippocampal S100B expression in a rat depression model. Behavioral and Brain Functions 2016; 12: 34.

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